

## WEST Search History

DATE: Monday, July 09, 2007

<u>Hide?</u>	<u>Set Name</u>	<u>Query</u>	<u>Hit Count</u>
<i>DB=PGPB; THES=ASSIGNEE; PLUR=YES; OP=ADJ</i>			
<input type="checkbox"/>	L9	L8 and (gly-266 or g266 or g-266)	3
<input type="checkbox"/>	L8	L7 and 266	79
<input type="checkbox"/>	L7	L5 and (variant or mut\$8)	532
<input type="checkbox"/>	L6	L1 and (variant or mut\$8)	0
<input type="checkbox"/>	L5	(lipase or phospholipase or lipolitic enzyme) and lanuginosa	621
<i>DB=USPT,USOC,EPAB,JPAB,DWPI; THES=ASSIGNEE; PLUR=YES; OP=ADJ</i>			
<input type="checkbox"/>	L4	L3 and (gly-266 or g266 or g-266)	0
<input type="checkbox"/>	L3	L2 and 266	70
<input type="checkbox"/>	L2	L1 and (variant or mut\$8)	659
<input type="checkbox"/>	L1	(lipase or phospholipase or lipolitic enzyme) and lanuginosa	988

END OF SEARCH HISTORY

## STN Search

10/779,427

FILE 'HOME' ENTERED AT 17:13:15 ON 09 JUL 2007

=&gt; file .nash

=&gt; s lanuginosa and (lipase or phospholipase or lipolytic enzyme) and (variant or mut?)

L1 24 FILE MEDLINE  
L2 77 FILE CAPLUS  
L3 61 FILE SCISEARCH  
L4 13 FILE LIFESCI  
L5 28 FILE BIOSIS  
L6 31 FILE EMBASE

TOTAL FOR ALL FILES

L7 234 LANUGINOSA AND (LIPASE OR PHOSPHOLIPASE OR LIPOLYTIC ENZYME)  
AND (VARIANT OR MUT?)

=&gt; s l7 and 266

L8 0 FILE MEDLINE  
L9 0 FILE CAPLUS  
L10 0 FILE SCISEARCH  
L11 0 FILE LIFESCI  
L12 0 FILE BIOSIS  
L13 0 FILE EMBASE

TOTAL FOR ALL FILES

L14 0 L7 AND 266

=&gt; s l7 and 263

L15 0 FILE MEDLINE  
L16 0 FILE CAPLUS  
L17 0 FILE SCISEARCH  
L18 0 FILE LIFESCI  
L19 0 FILE BIOSIS  
L20 0 FILE EMBASE

TOTAL FOR ALL FILES

L21 0 L7 AND 263

=&gt; d l7 1

L7 ANSWER 1 OF 234 MEDLINE on STN  
AN 2006408429 MEDLINE Full-text  
DN PubMed ID: 16784752  
TI Role of solvation barriers in protein kinetic stability.  
AU Rodriguez-Larrea David; Minning Stefan; Borchert Torben V; Sanchez-Ruiz Jose M  
CS Departamento de Quimica Fisica, Facultad de Ciencias, 18071-Granada, Spain.  
SO Journal of molecular biology, (2006 Jul 14) Vol. 360, No. 3, pp. 715-24.  
Electronic Publication: 2006-05-19.  
Journal code: 2985088R. ISSN: 0022-2836.  
CY England: United Kingdom  
DT Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)  
LA English  
FS Priority Journals  
EM 200608  
ED Entered STN: 11 Jul 2006  
Last Updated on STN: 17 Aug 2006  
Entered Medline: 16 Aug 2006

=&gt; s l7 not 2000-2007/py

L22 8 FILE MEDLINE  
L23 25 FILE CAPLUS  
L24 23 FILE SCISEARCH  
L25 8 FILE LIFESCI  
L26 10 FILE BIOSIS  
L27 12 FILE EMBASE

TOTAL FOR ALL FILES

L28 86 L7 NOT 2000-2007/PY

=> dup rem 128

PROCESSING COMPLETED FOR L28

L29 37 DUP REM L28 (49 DUPLICATES REMOVED)

=> d ibib abs 1-37

L29 ANSWER 1 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1999:231214 CAPLUS Full-text

DOCUMENT NUMBER: 130:264043

TITLE: Lipase variants and their use in  
detergent compositions

INVENTOR(S): Svendsen, Allan; Patkar, Shamkant Anant; Gormsen,  
Erik; Clausen, Ib Groth; Okkels, Jens Sigurd;  
Thellersen, Marianne

PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.

SOURCE: U.S., 50 pp., Cont.-in-part of U.S. Ser. No. 434,904,  
abandoned.

CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 5

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
-----	---	-----	-----	-----
US 5892013	A	19990406	US 1995-488271	19950905
CA 2092615	A1	19920314	CA 1991-2092615	19910913
PRIORITY APPLN. INFO.:			DK 1990-2194	A 19900913
			DK 1990-2195	A 19900913
			DK 1990-2196	A 19900913
			US 1993-977429	B1 19930222
			DK 1993-466	A 19930423
			DK 1994-217	A 19940222
			US 1995-434904	B2 19950501

AB The present invention relates to lipase variants which exhibit improved properties, detergent compns. comprising said lipase variants, DNA constructs coding for said lipase variants, and methods of making said lipase variants. The variants contain one or more deletions or substitutions of an amino acid residue in the lipid contact zone, or substitutions of two amino acid residues of the surface loop structure with cysteine which are positioned to form a disulfide bond. Numerous *Humicola lanuginosa* lipase variants were prepared with recombinant *Aspergillus oryzae*. Variants R209A+E210A, E56Q, D96L, D96K, D96W, and D96W+E210N had considerably better wash performance than the wild-type lipase. A number of the variants had higher specific activity and/or improved resistance to proteolytic degradation and almost all had improved resistance to alkaline conditions.

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 2 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1999:111576 CAPLUS Full-text

DOCUMENT NUMBER: 130:164901

TITLE: Lipase variants and their use in  
detergent compositions

INVENTOR(S): Svendsen, Allan; Patkar, Shamkant Anant; Gormsen,  
Erik; Okkels, Jens Sigurd; Thellersen, Marianne

PATENT ASSIGNEE(S): Novo Nordisk A/S, Norway

SOURCE: U.S., 51 pp., Cont.-in-part of U.S. Ser. No. 434,904,  
abandoned.

CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 5

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
-----	---	-----	-----	-----
US 5869438	A	19990209	US 1995-479275	19950607
CA 2092615	A1	19920314	CA 1991-2092615	19910913
PRIORITY APPLN. INFO.:			DK 1990-2194	A 19900913
			DK 1990-2195	A 19900913
			DK 1990-2196	A 19900913
			US 1991-977429	B1 19910913
			DK 1993-466	A 19930423
			DK 1994-217	A 19940222
			US 1995-434904	B2 19950501

AB The present invention relates to lipase variants which exhibit improved properties and to detergent additives and detergent compns. comprising said lipase variants. The variants contain one or more deletions or substitutions of an amino acid residue in the lipid contact zone, or substitutions of two amino acid residues of the surface loop structure with cysteine which are positioned to form a disulfide bond. Numerous Humicola lanuginosa lipase variants were prepared with recombinant Aspergillus oryzae. Variants R209A+E210A, E56Q, D96L, D96K, D96W, and D96W+E210N had considerably better wash performance than the wild-type lipase. A number of the variants had higher specific activity and/or improved resistance to proteolytic degradation and almost all had improved resistance to alkaline conditions.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 3 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:785630 SCISEARCH Full-text  
THE GENUINE ARTICLE: 246MN  
TITLE: Computational analysis of chain flexibility and fluctuations in Rhizomucor miehei lipase  
AUTHOR: Peters G H; Bywater R P (Reprint)  
CORPORATE SOURCE: Novo Nordisk AS, Biostruct Grp, DK-2760 Malov, Denmark (Reprint); Tech Univ Denmark, Dept Chem, DK-2800 Lyngby, Denmark  
COUNTRY OF AUTHOR: Denmark  
SOURCE: PROTEIN ENGINEERING, (SEP 1999) Vol. 12, No. 9, pp. 747-754.  
ISSN: 0269-2139.  
PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 77  
ENTRY DATE: Entered STN: 1999

Last Updated on STN: 1999

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB We have performed molecular dynamics simulation of Rhizomucor miehei lipase (Rml) with explicit water molecules present. The simulation was carried out in periodic boundary conditions and conducted for 1.2 ns in order to determine the concerted protein dynamics and to examine how well the essential motions are preserved along the trajectory. Protein motions are extracted by means of the essential dynamics analysis method for different lengths of the trajectory. Motions described by eigenvector 1 converge after approximately 200 ps and only small changes are observed with increasing simulation time. Protein dynamics along eigenvectors with larger indices, however, change with simulation time and generally, with increasing eigenvector index, longer simulation times are required for observing similar protein motions (along a particular eigenvector). Several regions in the protein show relatively large fluctuations and in particular motions in the active site lid and the segments Thr57-Asn63 and the active site hinge region Pro101-Gly104 are seen along several eigenvectors. These motions are generally associated with glycine residues, while no direct correlations are observed between these fluctuations and the positioning of prolines in the protein structure. The partial opening/closing of the lid is an example of induced fit mechanisms seen in other enzymes and could be a general mechanism for the activation of Rml.

L29 ANSWER 4 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:50858 SCISEARCH Full-text  
THE GENUINE ARTICLE: 155QB  
TITLE: Stereoselectivity of Mucorales lipases toward triacylglycerols - A simple solution to a complex problem  
AUTHOR: Scheib H; Pleiss J; Kovac A; Paltauf F; Schmid R D (Reprint)  
CORPORATE SOURCE: Univ Stuttgart, Inst Tech Biochem, Allmandring 31, D-70569 Stuttgart, Germany (Reprint); Univ Stuttgart, Inst Tech Biochem, D-70569 Stuttgart, Germany; Graz Tech Univ, Dept Biochem & Food Chem, A-8010 Graz, Austria  
COUNTRY OF AUTHOR: Germany; Austria  
SOURCE: PROTEIN SCIENCE, (JAN 1999) Vol. 8, No. 1, pp. 215-221.  
ISSN: 0961-8368.  
PUBLISHER: COLD SPRING HARBOR LAB PRESS, 1 BUNGTON RD, PLAINVIEW, NY 11724 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 21  
ENTRY DATE: Entered STN: 1999  
Last Updated on STN: 1999

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The lipases from *Rhizopus* and *Rhizomucor* are members of the family of *Mucorales* lipases. Although they display high sequence homology, their stereoselectivity toward triacylglycerols (sn-2 substituted triacylglycerols) varies. Four different triacylglycerols were investigated, which were classified into two groups: flexible substrates with rotatable O'-C1' ether or ester bonds adjacent to C2 of glycerol and rigid substrates with a rigid N'-C1' amide bond or a phenyl ring in sn-2. Although *Rhizopus* lipase shows opposite stereopreference for flexible and rigid substrates (hydrolysis in sn-1 and sn-3, respectively), *Rhizomucor* lipase hydrolyzes both groups of triacylglycerols preferably in sn-1. To explain these experimental observations, computer-aided molecular modeling was applied to study the molecular basis of stereoselectivity. A generalized model for both lipases of the *Mucorales* family highlights the residues mediating stereoselectivity: (1) L258, the C-terminal neighbor of the catalytic histidine, and (2) G266, which is located in a loop contacting the glycerol backbone of a bound substrate. Interactions with triacylglycerol substrates are dominated by van der Waals contacts. Stereoselectivity can be predicted by analyzing the value of a single substrate torsion angle that discriminates between sn-1 and sn-3 stereopreference for all substrates and lipases investigated here. This simple model can be easily applied in enzyme and substrate engineering to predict *Mucorales* lipase variants and synthetic substrates with desired stereoselectivity.

L29 ANSWER 5 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1999:287580 CAPLUS Full-text

DOCUMENT NUMBER: 131:84811

TITLE: Steady state fluorescence studies on the interaction of *Humicola lanuginosa* lipase with lipid vesicles

AUTHOR(S): Cajal, Y.; Prat, J.; Svendsen, A.; De Bolos, J.; Alsina, M. A.

CORPORATE SOURCE: Department of Physical Chemistry, School of Pharmacy, University of Barcelona, Barcelona, 08028, Spain

SOURCE: Biomedical Chromatography (1999), 13(2), 157-158  
CODEN: BICHE2; ISSN: 0269-3879

PUBLISHER: John Wiley & Sons Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB In this work, the conformational changes that take place in *Humicola lanuginosa* lipase (HLL) upon binding to vesicles of 1-palmitoyl-2-oleoylglycerol-sn-phosphoglycerol (POPG), and also in the presence of tributyrin (TB) as substrate, were studied by fluorescence techniques. The catalytic activity of the enzyme under the same conditions as used for fluorescence was measured spectroscopically by UV using p-nitrophenylbutyrate as substrate. An inactive mutant, with active site Ser-146 mutated to Ala, was used in the fluorescence expts. to avoid interference from product formation.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 6 OF 37 MEDLINE on STN DUPLICATE 1

ACCESSION NUMBER: 1998400942 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 9730809

TITLE: Active serine involved in the stabilization of the active site loop in the *Humicola lanuginosa* lipase.

AUTHOR: Peters G H; Svendsen A; Langberg H; Vind J; Patkar S A; Toxvaerd S; Kinnunen P K

CORPORATE SOURCE: Chemistry Department III, H.C. Orsted Institutet, University of Copenhagen, Denmark.. ghp@st.ki.ku.dk

SOURCE: Biochemistry, (1998 Sep 8) Vol. 37, No. 36, pp. 12375-83.  
Journal code: 0370623. ISSN: 0006-2960.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199902

ENTRY DATE: Entered STN: 23 Feb 1999

Last Updated on STN: 23 Feb 1999

Entered Medline: 5 Feb 1999

AB We have investigated the binding properties of and dynamics in *Humicola lanuginosa* lipase (Hll) and the inactive mutant S146A (active Ser146 substituted with Ala) using fluorescence spectroscopy and molecular dynamics simulations, respectively. Hll and S146A show significantly different binding behavior for phosphatidylcholine (PC) and phosphatidylglycerol (PG) liposomes. Generally, higher binding affinity is observed for Hll than the S146A mutant. Furthermore, depending on the matrix, the addition of the transition state analogue benzene boronic acid increases the binding affinity of S146A, whereas only small changes are observed for Hll suggesting that the active site

lid in the latter opens more easily and hence more lipase molecules are bound to the liposomes. These observations are in agreement with molecular dynamics simulations and subsequent essential dynamics analyses. The results reveal that the hinges of the active site lid are more flexible in the wild-type H11 than in S146A. In contrast, larger fluctuations are observed in the middle region of the active site loop in S146A than in H11. These findings reveal that the single mutation (S146A) of the active site serine leads to substantial conformational alterations in the H. lanuginosa lipase and different binding affinities.

L29 ANSWER 7 OF 37 MEDLINE on STN DUPLICATE 2  
 ACCESSION NUMBER: 1998244839 MEDLINE Full-text  
 DOCUMENT NUMBER: PubMed ID: 9578545  
 TITLE: Interfacial activation of triglyceride lipase from Thermomyces (Humicola) lanuginosa: kinetic parameters and a basis for control of the lid.  
 AUTHOR: Berg O G; Cajal Y; Butterfoss G L; Grey R L; Alsina M A; Yu B Z; Jain M K  
 CORPORATE SOURCE: Department of Chemistry and Biochemistry, University of Delaware, Newark 19716, USA.  
 CONTRACT NUMBER: GM29703 (NIGMS)  
 SOURCE: Biochemistry, (1998 May 12) Vol. 37, No. 19, pp. 6615-27. Journal code: 0370623. ISSN: 0006-2960.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: (COMPARATIVE STUDY)  
 Journal; Article; (JOURNAL ARTICLE)  
 (RESEARCH SUPPORT, NON-U.S. GOV'T)  
 (RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199806  
 ENTRY DATE: Entered STN: 11 Jun 1998  
 Last Updated on STN: 29 Jan 1999  
 Entered Medline: 4 Jun 1998

AB A strategy is developed to analyze steady-state kinetics for the hydrolysis of a soluble substrate partitioned into the interface by an enzyme at the interface. The feasibility of this approach to obtain interfacial primary kinetic and equilibrium parameters is demonstrated for a triglyceride lipase. Analysis for phospholipase A2 catalyzed hydrolysis of rapidly exchanging micellar (Berg et al. (1997) Biochemistry 36, 14512-14530) and nonexchangeable vesicular (Berg et al., (1991) Biochemistry 30, 7283-7291) phospholipids is extended to include the case of a substrate that does not form the interface. The triglyceride lipase (tLTGL) from Thermomyces (formerly Humicola) lanuginosa hydrolyzes p-nitrophenylbutyrate or tributyrin partitioned in the interface of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) vesicles at a rate that is more than 100-fold higher than that for the monodispersed substrate or for the substrate partitioned into zwitterionic vesicles. Catalysis and activation is not seen with the S146A mutant without the catalytic serine-146; however, it binds to the POPG interface with the same affinity as the WT. Thus POPG acts as a diluent surface to which the lipase binds in an active, or "open", form for the catalytic turnover; however, the diluent molecules have poor affinity for the active site. Analysis of the substrate and the diluent concentration dependence of the rate of hydrolysis provides a basis for the determination of the primary interfacial catalytic parameters. As a competitive substrate, tributyrin provided a check for the apparent affinity parameters. Nonidealities from the fractional difference in the molecular areas in interfaces are expressed as the area correction factor and can be interpreted as a first-order approximation for the interfacial activity coefficient. The basis for the interfacial activation of tLTGL on anionic interface is attributed to cationic R81, R84, and K98 in the "hinge" around the 86-93 "lid" segment of tLTGL.

L29 ANSWER 8 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 3  
 ACCESSION NUMBER: 1998:711158 CAPLUS Full-text  
 DOCUMENT NUMBER: 130:92064  
 TITLE: Spectroscopic studies of the interfacial binding of Humicola lanuginosa lipase  
 AUTHOR(S): Cajal, Yolanda; Prat, Josefina; De Bolos, Jordi; Asuncion Alsina, M.; Svendsen, Allan  
 CORPORATE SOURCE: School of Pharmacy, Department of Physical Chemistry, University of Barcelona, Barcelona, 08028, Spain  
 SOURCE: Analyst (Cambridge, United Kingdom) (1998), 123(11), 2229-2233  
 CODEN: ANALAO; ISSN: 0003-2654  
 PUBLISHER: Royal Society of Chemistry  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB The interaction of Humicola lanuginosa lipase (HLL) with small unilamellar vesicles of 1-palmitoyl-2-oleoylglycero-sn-3-phosphoglycerol (POPG) and in the presence of tributyrin (TB) as a substrate was studied by the use of steady-state fluorescence techniques. An inactive mutant with

the serine from the catalytic triad changed by alanine (S146A) was used in expts. with TB to avoid interferences from product formation. HLL binds to POPG vesicles in an active or open form for the catalytic turnover, therefore POPG provides a suitable system for studying the conformational changes involving the movement of the loop of amino acids that covers the active site of the enzyme in solution. Tryptophan (Trp) fluorescence shows that HLL binding to POPG occurs with a change in the environment of Trp residue(s) and that there is only one type of bound form, even in the presence of TB. Accessibility to aqueous quenchers indicates shielding of Trp in the membrane. Fluorescence anisotropy of the enzyme increases on binding to the vesicles, indicating restricted rotational freedom for the Trp due to penetration in the bilayer. Resonance energy transfer expts. using an interfacial membrane probe, 1-[4-(trimethylammonio)phenyl]-6-phenylhexa-1,3,5-triene p-toluenesulfonate (TMA-DPH), and an internal membrane probe, 1,6-diphenylhexa-1,3,5-triene (DPH), indicate that HLL does not penetrate very deeply in the hydrophobic core of the membrane, but preferentially stays close to the lipid interface. Addition of substrate (TB) does not result in any addnl. changes in the spectroscopic properties of HLL. It is suggested that the observed changes are due to the 'opening of the lid' on binding to POPG vesicles, leaving the active site accessible for the substrate to bind.

REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 9 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1998:229525 SCISEARCH Full-text

THE GENUINE ARTICLE: ZC005

TITLE: A proposed architecture for lecithin cholesterol acyl  
transferase (LCAT): Identification of the catalytic triad  
and molecular modeling

AUTHOR: Peelman F; Vinaimont N; Verhee A; Vanloo B; Verschelde J  
L; Labeur C; Seguret-Mace S; Duverger N; Hutchinson G;  
Vandekerckhove J; Tavernier J; Rosseneu M (Reprint)

CORPORATE SOURCE: Univ Ghent, Dept Biochem, Lab Lipoprot Chem, Hosp Str 13,  
B-9000 Ghent, Belgium (Reprint); Univ Ghent, Dept Biochem,  
Lab Lipoprot Chem, B-9000 Ghent, Belgium; State Univ Ghent  
VIB, Fac Med, Dept Biochem, B-9000 Ghent, Belgium; Rhone  
Poulenc Rorer, GENCELL, Cardiovasc Dept, Vitry Sur Seine,  
France; Univ Coll London, Dept Biochem, London, England  
maryvonne.rosseneu@rug.ac.be

COUNTRY OF AUTHOR: Belgium; France; England

SOURCE: PROTEIN SCIENCE, (MAR 1998) Vol. 7, No. 3, pp. 587-599.  
ISSN: 0961-8368.

PUBLISHER: COLD SPRING HARBOR LAB PRESS, PUBLICATIONS DEPT, 500  
SUNNYSIDE BLVD, WOODBURY, NY 11797-2924 USA.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 52

ENTRY DATE: Entered STN: 1998

Last Updated on STN: 15 Mar 2007

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The enzyme cholesterol lecithin acyl transferase (LCAT) shares the Ser/Asp-Glu/His triad with lipases, esterases and proteases, but the low level of sequence homology between LCAT and these enzymes did not allow for the LCAT fold to be identified yet. We, therefore, relied upon structural homology calculations using threading methods based on alignment of the sequence against a library of solved three-dimensional protein structures, for prediction of the LCAT fold. We propose that LCAT, like lipases, belongs to the alpha/beta hydrolase fold family, and that the central domain of LCAT consists of seven conserved parallel beta-strands connected by four alpha-helices and separated by loops. We used the conserved features of this protein fold for the prediction of functional domains in LCAT, and carried out site-directed mutagenesis for the localization of the active site residues. The wild-type enzyme and mutants were expressed in Cos-1 cells. LCAT mass was measured by ELISA, and enzymatic activity was measured on recombinant HDL, on LDL and on a monomeric substrate. We identified D345 and H377 as the catalytic residues of LCAT, together with F103 and L182 as the oxyanion hole residues. In analogy with lipases, we further propose that a potential "lid" domain at residues 50-74 of LCAT might be involved in the enzyme-substrate interaction. Molecular modeling of human LCAT was carried out using human pancreatic and *Candida antarctica* lipases as templates. The three-dimensional model proposed here is compatible with the position of natural mutants for either LCAT deficiency or Fish-eye disease. It enables moreover prediction of the LCAT domains involved in the interaction with the phospholipid and cholesterol substrates.

L29 ANSWER 10 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 4

ACCESSION NUMBER: 1998:811251 CAPLUS Full-text

DOCUMENT NUMBER: 130:164804

TITLE: Fluorescence study of fungal lipase from  
*Humicola lanuginosa*

AUTHOR(S): Stobiecka, A.; Wysocki, S.; Brzozowski, A. M.

CORPORATE SOURCE: Inst. General Food Chem., Tech. Univ., Lodz, 90-924,  
Pol.  
SOURCE: Journal of Photochemistry and Photobiology, B: Biology  
(1998), 45(2-3), 95-102  
CODEN: JPPBEG; ISSN: 1011-1344  
PUBLISHER: Elsevier Science S.A.  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Time-resolved and steady-state fluorescence quenching measurements have been performed to study two different conformations of the fungal lipase from *Humicola lanuginosa*. The intrinsic fluorescence of tryptophan Trp89 residue, located in the 'lid' region, has been used as a probe for the dynamics of protein. The native ('closed-lid') form of the enzyme has been found to decay as a triple exponential with time consts. and relative contributions of 5.4 ns (74.3%), 2.2 ns (20.4%) and 0.4 ns (5.3%). A comparison of recovered decay parameters obtained for native and mutated *H. lanuginosa* lipase shows that Trp89 contributes about 61% to the class of emitting species with the lifetime of 5.4 ns. The fluorescence quenching data show that three out of four tryptophans (i.e., 117, 221 and 260 residues) within *H. lanuginosa* lipase are totally quenchable by acrylamide while completely inaccessible to iodide. On the contrary, the Trp89 residue is available for both quenchers. Using steady-state iodide fluorescence quenching data and the fluorescence-quenching-resolved-spectra (FQRS) method, the total emission spectrum of the native lipase has been decomposed into two spectral components. One of them, unquenchable by iodide, has a maximum of fluorescence emission at 330 nm and the second one, exposed to the solvent, emits at 338 nm. The resolved spectrum of the redder component corresponds to the Trp89 residue, which participates in about 65% of the total *H. lanuginosa* emission. The dynamic Stern-Volmer quenching consts. calculated for both native ('closed-lid') and inhibited ('open-lid') lipase are 2.71 and 4.49 M<sup>-1</sup>, resp. The values obtained indicate that Trp89 is not deeply buried in the protein matrix. Our results suggest that distinct configurations of fungal lipase can be monitored using the fluorescence of the Trp89 residue located in the 'lid'-helix which participates in an interfacial activation of the enzyme.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 11 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1998:587613 SCISEARCH Full-text  
THE GENUINE ARTICLE: 106NT  
TITLE: Anatomy of lipase binding sites: the scissile  
fatty acid binding site  
AUTHOR: Pleiss J; Fischer M; Schmid R D (Reprint)  
CORPORATE SOURCE: Univ Stuttgart, Inst Tech Biochem, Allmandring 31, D-70569  
Stuttgart, Germany (Reprint); Univ Stuttgart, Inst Tech  
Biochem, D-70569 Stuttgart, Germany  
COUNTRY OF AUTHOR: Germany  
SOURCE: CHEMISTRY AND PHYSICS OF LIPIDS, (JUN 1998) Vol. 93, No.  
1-2, pp. 67-80.  
ISSN: 0009-3084.  
PUBLISHER: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY  
15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 41  
ENTRY DATE: Entered STN: 1998  
Last Updated on STN: 1998

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Shape and physico-chemical properties of the scissile fatty acid binding sites of six lipases and two serine esterases were analyzed and compared in order to understand the molecular basis of substrate specificity. All eight serine esterases and lipases have similar architecture and catalytic mechanism of ester hydrolysis, but different substrate specificities for the acyl moiety. Lipases and esterases differ in the geometry of their binding sites, lipases have a large, hydrophobic scissile fatty acid binding site, esterases like acetylcholinesterase and bromoperoxidase have a small acyl binding pocket, which fits exactly to their favorite substrates. The lipases were subdivided into three sub-groups: (1) lipases with a hydrophobic, crevice-like binding site located near the protein surface (lipases from *Rhizomucor* and *Rhizopus*); (2) lipases with a funnel-like binding site (lipases from *Candida antarctica*, *Pseudomonas* and mammalian pancreas and cutinase); and (3) lipases with a tunnel-like binding site (lipase from *Candida rugosa*). The length of the scissile fatty acid binding site varies considerably among the lipases between 7.8 Angstrom in cutinase and 22 Angstrom in *Candida rugosa* and *Rhizomucor miehei* lipase. Location and properties of the scissile fatty acid binding sites of all lipases of known structure were characterized. Our model also identifies the residues which mediate chain length specificity and thus may guide protein engineering of lipases for changed chain length specificity. The model was supported by published experimental data on the chain length specificity profile of various lipases and on mutants of fungal lipases with changed fatty acid chain length specificity. (C) 1998 Elsevier Science Ireland Ltd. All rights reserved.



L29 ANSWER 12 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1998:587612 SCISEARCH Full-text  
THE GENUINE ARTICLE: 106NT  
TITLE: Insights into the molecular basis for fatty acyl  
specificities of lipases from *Geotrichum*  
*candidum* and *Candida rugosa*  
AUTHOR: Holmquist M (Reprint)  
CORPORATE SOURCE: Royal Inst Technol, Dept Biochem & Biotechnol, SE-10044  
Stockholm, Sweden (Reprint)  
COUNTRY OF AUTHOR: Sweden  
SOURCE: CHEMISTRY AND PHYSICS OF LIPIDS, (JUN 1998) Vol. 93, No.  
1-2, pp. 57-66.  
ISSN: 0009-3084.  
PUBLISHER: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY  
15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 49  
ENTRY DATE: Entered STN: 1998  
Last Updated on STN: 1998

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Despite immense progress in our comprehension of lipase structure and function during the past decade, the basis for lipase acyl specificities has remained poorly understood. This review summarizes some recent advances in the understanding at the molecular-level of substrate acyl recognition by two members in a group of large (M-w similar to 60 kDa) microbial lipases. Two aspects of acyl specificity will be focused upon. (i) The unique preference of a fungal *Geotrichum candidum* lipase for long-chain cis ( $\Delta^9$ ) unsaturated fatty acid moieties in the substrate. Mutational analysis of this lipase identified residues essential for its anomalous acyl preference. This information highlighted for the first time parts in the lipase molecule involved in substrate acyl differentiation. These results are discussed in the context of the 3D-structure of a *G. candidum* lipase isoenzyme and structures of the related *Candida rugosa* lipase in complex with inhibitors. (ii) The mechanism by which the yeast *C. rugosa* lipase discriminates between enantiomers of a substrate with a chiral acyl moiety. Molecular modeling in combination with substrate engineering and kinetic analyses, identified two alternative substrate binding modes. This allowed for the proposal of a molecular mechanism explaining how long-chain alcohols can act as enantioselective inhibitors of this enzyme. A picture is thus beginning to emerge of the interplay between lipase structure and fatty acyl specificity. (C) 1998 Elsevier Science Ireland Ltd. All rights reserved.

L29 ANSWER 13 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 5

ACCESSION NUMBER: 1998:455633 CAPLUS Full-text  
DOCUMENT NUMBER: 129:213493  
TITLE: The decay of the fluorescence anisotropy of tryptophan  
residues in fungal lipase from *Humicola*  
*lanuginosa*  
AUTHOR(S): Stobiecka, A.; Wysocki, S.  
CORPORATE SOURCE: Institute General Food Chemistry, Technical University  
Lodz, Lodz, 90924, Pol.  
SOURCE: Journal of Radioanalytical and Nuclear Chemistry  
(1998), 232(1-2), 43-48  
CODEN: JRNCDE; ISSN: 0236-5731  
PUBLISHER: Elsevier Science S.A.  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Pulse nanosecond fluorescence anisotropy decay was used to study the mobility of Trp residues within fungal lipase from *Humicola lanuginosa*. The decay of emission anisotropy of protein in native, inhibited, and mutated form was investigated in buffered water and 50% volume/volume glycerol solns. The rotational motions of the lipase were analyzed in terms of 2 different kinetic models. The fluorescence emission anisotropy decay can best be described with 2 rotational correlation times: 0.63 and 5.45 ns in water and 0.98 and 10.70 ns and in 50% volume/volume glycerol solution. The decay of inhibited and mutated *H. lanuginosa* lipase showed a similar biexponential character. These results are interpreted in terms of local or segmental motion arising from a mass of about 1083 Da which corresponds to the 'lid'-helix fragment of the enzyme.

L29 ANSWER 14 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1998:587609 SCISEARCH Full-text  
THE GENUINE ARTICLE: 106NT

TITLE: Biochemical properties of staphylococcal (phospho) lipases

AUTHOR: Simons J W F A; Gotz F; Egmond M R; Verheij H M (Reprint)

CORPORATE SOURCE: Univ Utrecht, Inst Biomembranes, Ctr biomembranes & Lipid Enzymol, Dept Enzymol & Prot Engn, POB 80054, Padualaan 8, NL-3508 TB Utrecht, Netherlands (Reprint); Univ Utrecht, Inst Biomembranes, Ctr biomembranes & Lipid Enzymol, Dept Enzymol & Prot Engn, NL-3508 TB Utrecht, Netherlands; Univ Tubingen, Tubingen, Germany

COUNTRY OF AUTHOR: Netherlands; Germany

SOURCE: CHEMISTRY AND PHYSICS OF LIPIDS, (JUN 1998) Vol. 93, No. 1-2, pp. 27-37.  
ISSN: 0009-3084.

PUBLISHER: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 37

ENTRY DATE: Entered STN: 1998  
Last Updated on STN: 1998

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Various staphylococci secrete lipases which require calcium ions for activity, and have profound preferences for substrates with different chain lengths. The lipase from *Staphylococcus hyicus* is exceptional since it has higher phospholipase than lipase activity. This paper gives an overview of the biochemical properties of these enzymes. It appears that chain length selectivity of these enzymes resides in the acylation step. Interfaces mainly influence the acylation step. Calcium ions do not influence the rate of acylation or deacylation although stabilise the enzyme against denaturation. Molecular modelling based on the X-ray structure of *Pseudomonas glumae* lipase was used to construct a model of the staphylococcal lipases. With this model the position of several residues involved in substrate selectivity was predicted. Moreover, a sequence element could be assigned that may function as the so-called lid domain in staphylococcal lipases. Sequence alignment of four staphylococcal lipases, and lipases from *P. glumae* and *Bacillus thermocatenulatus* identified several potential calcium ligands, one of which was verified by site directed mutagenesis. It is concluded that stabilisation of lipases by calcium ions might be a more general phenomenon than recognized so far. (C) 1998 Elsevier Science Ireland Ltd. All rights reserved.

L29 ANSWER 15 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1997:757085 CAPLUS Full-text

DOCUMENT NUMBER: 128:24317

TITLE: Detergent compositions containing a specific lipolytic enzyme and alkyl polyglycoside surfactant

INVENTOR(S): Baeck, Andre Cesar; Kasturi, Chandrika

PATENT ASSIGNEE(S): Procter and Gamble Company, USA; Baeck, Andre Cesar; Kasturi, Chandrika

SOURCE: PCT Int. Appl., 74 pp.  
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9743377	A1	19971120	WO 1996-US7088	19960515
W:	AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
AU 9658618	A	19971205	AU 1996-58618	19960515
EP 912683	A1	19990506	EP 1996-920251	19960515
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI			
BR 9612608	A	19990720	BR 1996-12608	19960515
PRIORITY APPLN. INFO.:			WO 1996-US7088	W 19960515

AB The present invention relates to detergent compns. containing a variants of the native lipolytic enzyme producible by *Humicola lanuginosa* and *Thermomyces lanuginosus* or by cloning and expressing the gene responsible for producing the variants into a host organism and an ROGx surfactant (R = C10-16 alkyl, G = reducing saccharide containing 5-6 C atoms, x = 1-3). Such compns. provide

improved overall detergency performance: reduced redeposition of greasy/oily substances on fabrics, dishware and hard surfaces thereby improving whiteness maintenance, cleaning, spotting, filming and stain removal performances.

L29 ANSWER 16 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1997:757082 CAPLUS Full-text

DOCUMENT NUMBER: 128:36417

TITLE: Detergent compositions comprising specific lipolytic enzyme and a soil release polymer

INVENTOR(S): Baeck, Andre Cesar; Kasturi, Chandrika

PATENT ASSIGNEE(S): Procter and Gamble Company, USA; Baeck, Andre Cesar; Kasturi, Chandrika

SOURCE: PCT Int. Appl., 77 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9743374	A1	19971120	WO 1996-US7085	19960515
W:	AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
CA 2254919	A1	19971120	CA 1996-2254919	19960515
AU 9657957	A	19971205	AU 1996-57957	19960515
BR 9612614	A	19990720	BR 1996-12614	19960515
EP 931134	A1	19990728	EP 1996-914666	19960515

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI

PRIORITY APPLN. INFO.: WO 1996-US7085 A 19960515

AB Lipolytic enzymes are variants of the native lipolytic enzymes producible by *Humicola lanuginosa* and *Thermomyces lanuginosus*. A soil release polymer is selected from sulfonated poly(ethoxy)/(propoxy)-end capped ester oligomers and/or short block polymers manufactured from di-Me terephthalate (I), 1,2-propylene glycol (II), Me-capped polyethylene glycol and/or short block polymers manufactured from I, II, and sulfoethoxylates. Thus, a granular detergent contained a Na linear C12-alkyl benzenesulfonate 22, a phosphate 23, a silicate 14, Zeolite A 8.2, Dequest 2060 0.4, Na sulfate 5.5, amylase 0.005, protease 0.01, pectinase 0.02, Lipolase Ultra 0.005, a soil release polymer 0.2, cellulase 0.001, and water and minors to 100%.

L29 ANSWER 17 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1997:220628 CAPLUS Full-text

DOCUMENT NUMBER: 126:208956

TITLE: Recombinant lipases with C- and/or

N-terminal extensions and their use in detergents

INVENTOR(S): Fuglsang, Claus Crone; Okkels, Jens Sigurd; Pertersen, Dorte Aaby; Patkar, Shamkant Anant; Thellersen, Marianne; Vind, Jesper; Halkier, Torben; Joergensen, Steen Troels; et al.

PATENT ASSIGNEE(S): Novo Nordisk A/s, Den.; Fuglsang, Claus Crone; Okkels, Jens Sigurd; Pertersen, Dorte Aaby; Patkar, Shamkant Anant; Thellersen, Marianne; Vind, Jesper; Halkier, Torben

SOURCE: PCT Int. Appl., 197 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 4

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9704078	A1	19970206	WO 1996-DK321	19960712
W:	AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG			

RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR,  
IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA

AU 9664140 A 19970218 AU 1996-64140 19960712  
CN 1193346 A 19980916 CN 1996-196371 19960712  
PRIORITY APPLN. INFO.: DK 1995-832 A 19950714  
DK 1995-1013 A 19950913  
DK 1995-1096 A 19950929  
DK 1995-1306 A 19951121  
US 1996-11634P P 19960214  
DK 1996-372 A 19960401  
US 1996-20461P P 19960507  
WO 1996-DK321 W 19960712

AB The invention relates to a modified enzyme with lipolytic activity recovered from a filamentous fungi or a bacteria having one or more peptide addns. at the N-terminal and/or the C-terminal ends in comparison to the parent enzyme. The peptide addns. significantly improve the washing performance of the lipase. Further, the invention relates to a DNA sequence encoding said modified enzyme, a vector comprising said DNA sequence, a host cell harboring said DNA sequence or said vector, and a process for producing said modified enzyme with lipolytic activity. The lipase variants are useful in detergent comps. Numerous lipase variants containing substitution mutations and C- and/or N-terminal addns. were prepared with recombinant *Aspergillus oryzae* or with *Escherichia coli*. The addition of SPIRR to the N-terminus of *Humicola lanuginosa* lipase increased the wash performance relative to the parent enzyme twofold.

L29 ANSWER 18 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1997:897558 SCISEARCH Full-text  
THE GENUINE ARTICLE: YJ709  
TITLE: Identification of residues essential for differential  
fatty acyl specificity of *Geotrichum candidum*  
lipases I and II  
AUTHOR: Holmquist M (Reprint); Tessier D C; Cygler M  
CORPORATE SOURCE: NATL RES COUNCIL CANADA, BIOTECHNOL RES INST, MONTREAL, PQ  
H4P 2R2, CANADA; ROYAL INST TECHNOL, DEPT BIOCHEM &  
BIOTECHNOL, S-10044 STOCKHOLM, SWEDEN  
COUNTRY OF AUTHOR: CANADA; SWEDEN  
SOURCE: BIOCHEMISTRY, (2 DEC 1997) Vol. 36, No. 48, pp.  
15019-15025.  
ISSN: 0006-2960.  
PUBLISHER: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 44  
ENTRY DATE: Entered STN: 1997  
Last Updated on STN: 1997

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The fungus *Geotrichum candidum* produces two lipase isoenzymes, GCL I and GCL II, with distinct differences in substrate specificity despite their 86% identical primary structure, GCL I prefers ester substrates with long-chain cis (Delta-9) unsaturated fatty acid moieties, whereas GCL II also accepts medium-length (C8-C14) acyl moieties in the substrate. To reveal structural elements responsible for differences in substrate differentiating ability of these isoenzymes, we designed, expressed, and characterized 12 recombinant lipase variants. Three chimeric lipases containing unique portions of the N-terminal and the C-terminal part of GCL I and GCL II, respectively, were constructed and enzymatically characterized. Activities were measured against mixed triglyceride-poly(dimethyl siloxane) particles. Our results indicate that residues within sequence positions 349-406 are essential for GCL I's high triolein/trioctanoin activity ratio of 20. The substitution of that segment in the specific GCL I to the corresponding residues in the nonspecific GCL II, resulted in an enzyme with a triolein/trioctanoin activity ratio of 1.4, identical to that of GCL II. The reverse mutation in GCL II increased its specificity for triolein by a factor of 2, thus only in part restoring the high specificity seen with GCL I. In further experiments, the point mutations at the active site entrance of the GCL I, Leu358Phe and Ile357Ala/Leu358Phe, lowered the triolein/trioctanoin activity ratio from 20 to 4 and 2.5, respectively. The substitutions Cys379Phe/Ser380Tyr at the bottom of the active site cavity of GCL I decreased its specificity to a value of 3.6. Measurements of lipase activity with substrate particles composed of pure triglycerides or ethyl esters of oleic and octanoic acids resulted in qualitatively similar results as reported above. Our data reveal for the first time the identity of residues essential for the unusual substrate preference of GCL I and show that the anatomy, both at the entrance and the bottom of the active site cavity, plays a key role in substrate discrimination.

L29 ANSWER 19 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 6  
ACCESSION NUMBER: 1997:372120 CAPLUS Full-text

DOCUMENT NUMBER: 126:327220  
TITLE: Comparison of the adsorption and activity of  
lipases from *Humicola lanuginosa* and  
*Candida antarctica* on solid surfaces  
AUTHOR(S): Wannerberger, Kristin; Arnebrant, Thomas  
CORPORATE SOURCE: Department of Food Technology, University of Lund,  
Lund, S-221 00, Swed.  
SOURCE: Langmuir (1997), 13(13), 3488-3493  
CODEN: LANGD5; ISSN: 0743-7463  
PUBLISHER: American Chemical Society  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The adsorption of lipases from *H. lanuginosa*, the wild type (WT) and a mutant with increased hydrophobicity in the active site region, and lipase B from *C. antarctica* to solid surfaces was studied by in situ ellipsometry. In addition, the activity of the adsorbed lipase was measured in situ and from the different surface concns., the specific activity was calculated. Concentration- and temperature-dependence as well as the influence of surface wettability was studied using silica surfaces with varying degrees of methylation. The higher hydrophobicity of the mutant compared to the WT resulted in increased amts. adsorbed, no desorbable fraction during rinsing, and absence of an initial maximum in adsorbed amount (as seen for the WT) at higher concns. No temperature dependence for the mutant could be observed. This was in contrast to the WT where both the plateau value of the adsorbed amount and the activity decreased with increasing temperature. The influence of surface wettability was similar for both featuring a decreased adsorbed amount and increased specific activity with increasing wettability. The amount adsorbed of the *Candida* lipase was significantly higher at all concns., compared to the other lipases, and the activity was very low, indicating adsorption with the active site region directed toward the surface. The surface wettability did not affect the activity of *Candida* lipase.

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 20 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1997:548646 CAPLUS Full-text  
DOCUMENT NUMBER: 127:189671  
TITLE: Biotechnology in detergents and cosmetics  
AUTHOR(S): Ito, Susumu  
CORPORATE SOURCE: Tochigi Res. Lab., Kao Corp., Tochigi, 321-34, Japan  
SOURCE: Baioisaiensu to Indasutori (1997), 55(8), 541-545  
CODEN: BIDSE6; ISSN: 0914-8981  
PUBLISHER: Baioindasutori Kyokai  
DOCUMENT TYPE: Journal; General Review  
LANGUAGE: Japanese

AB A review with 18 refs. on site-directed mutation of subtilisin suitable for detergents, heat-stable mutation of *Bacillus licheniformis* amylase, amylopullulanase, site-directed mutation of  $\alpha$ -amylase to improve durability against chelating agents and heat, alkaline cellulase of alkalophilic *Bacillus*, cellulase of *Aspergillus oryzae*, production of lipase of *Humicola lanuginosa* by *A. oryzae* as host organism, catalytic site of *Mocor miehei* enzyme, raw materials for cosmetics, sophorolipid, kojic acid, hyaluronate,  $\gamma$ -linolenate, shikonin, and tuberose polysaccharide.

L29 ANSWER 21 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1997:250761 SCISEARCH Full-text  
THE GENUINE ARTICLE: WP714  
TITLE: Computational studies of the activation of lipases  
and the effect of a hydrophobic environment  
AUTHOR: Peters G H (Reprint); Toxvaerd S; Olsen O H; Svendsen A  
CORPORATE SOURCE: UNIV COPENHAGEN, HC ORSTED INST, DEPT CHEM 3, UNIV PK 5,  
DK-2100 COPENHAGEN O, DENMARK (Reprint); NOVO NORDISK AS,  
DK-2880 BAGSVAERD, DENMARK  
COUNTRY OF AUTHOR: DENMARK  
SOURCE: PROTEIN ENGINEERING, (FEB 1997) Vol. 10, No. 2, pp.  
137-147.  
ISSN: 0269-2139.  
PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD, ENGLAND OX2  
6DP.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 69  
ENTRY DATE: Entered STN: 1997  
Last Updated on STN: 1997

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB We have investigated the activation pathway of three wild type lipases and three mutants using molecular dynamics techniques combined with a constrained mechanical protocol. The

activation of these lipases involves a rigid body hinge-type motion of a single helix, which is displaced during activation to expose the active site and give access to the substrate. Our results suggest that the activation of lipases is enhanced in a hydrophobic environment as is generally observed in experiments. The energy gain upon activation varies between the different lipases and depends strongly on the distribution of the charged residues in the activating loop region. In a low dielectric constant medium (such as a lipid environment), the electrostatic interactions between the residues located in the vicinity of the activating loop (lipid contact zone) are dominant and determine the activation of the lipases. Calculations of the pK(a)s qualitatively indicate that some titratable residues experience significant pK shifts upon activation. These calculations may provide sufficient details for an understanding of the origin and magnitude of a given electrostatic effect and may provide an avenue for exploring the activation pathway of lipases.

L29 ANSWER 22 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1997:170960 SCISEARCH Full-text  
THE GENUINE ARTICLE: WJ876  
TITLE: Altered acyl chain length specificity of Rhizopus delemar  
lipase through mutagenesis and molecular  
modeling  
AUTHOR: Klein R R (Reprint); King G; Moreau R A; Haas M J  
CORPORATE SOURCE: USDA ARS, ERRC, 600 E MERMAID LANE, WYNDMOOR, PA 19038  
(Reprint)  
COUNTRY OF AUTHOR: USA  
SOURCE: LIPIDS, (FEB 1997) Vol. 32, No. 2, pp. 123-130.  
ISSN: 0024-4201.  
PUBLISHER: AMER OIL CHEMISTS SOC, 1608 BROADMOOR DRIVE, CHAMPAIGN, IL  
61821-0489.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE; AGRI  
LANGUAGE: English  
REFERENCE COUNT: 27  
ENTRY DATE: Entered STN: 1997  
Last Updated on STN: 1997

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The acyl binding site of Rhizopus delemar prolipase and mature lipase was altered through site-directed mutagenesis to improve lipase specificity for short- or medium-chain length fatty acids. Computer-generated structural models of R. delemar lipase were used in mutant protein design and in the interpretation of the catalytic properties of the resulting recombinant enzymes. Molecular dynamics simulations of the double mutant, val209trp + phe112trp, predicted that the introduction of trp112 and trp209 in the acyl binding groove would sterically hinder the docking of fatty acids longer than butyric acid. Assayed against a mixture of triacylglycerol substrates, the val209trp + phe112trp mature lipase mutant showed an 80-fold increase in the hydrolysis of tributyrin relative to the hydrolysis of tricaprylin while no triolein hydrolysis was detected. By comparison, the val94Trp mutant, predicted to pose steric or geometric constraints for docking fatty acids longer than caprylic acid in the acyl binding groove, resulted in a modest 1.4-fold increase in tricaprylin hydrolysis relative to the hydrolysis of tributyrin. Molecular models of the double mutant phe95asp + phe214arg indicated the creation of a salt bridge between asp95 and arg214 across the distal end of the acyl binding groove. When challenged with a mixture of triacylglycerols, the phe95asp + phe214arg substitutions resulted in an enzyme with 3-fold enhanced relative activity for tricaprylin compared to triolein, suggesting that structural determinants for medium-chain length specificity may reside in the distal end of the acyl binding groove. Attempts to introduce a salt bridge within 8 Angstrom of the active site by the double mutation leu146lys + ser115asp destroyed catalytic activity entirely. Similarly, the substitution of polar Gln at the rim of the acyl binding groove for phe112 largely eliminated catalytic activity of the lipase.

L29 ANSWER 23 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1997:470292 SCISEARCH Full-text  
THE GENUINE ARTICLE: XF115  
TITLE: Computer-aided modelling of stereoselective triglyceride  
hydrolysis catalyzed by Rhizopus oryzae lipase  
AUTHOR: Holzwarth H C (Reprint); Pleiss J; Schmid R D  
CORPORATE SOURCE: UNIV STUTTGART, INST TECH BIOCHEM, D-70569 STUTTGART,  
GERMANY  
COUNTRY OF AUTHOR: GERMANY  
SOURCE: JOURNAL OF MOLECULAR CATALYSIS B-ENZYMATIC, (10 JUN 1997)  
Vol. 3, No. 1-4, pp. 73-82.  
ISSN: 1381-1177.  
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM,

NETHERLANDS.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: PHYS  
LANGUAGE: English  
REFERENCE COUNT: 28  
ENTRY DATE: Entered STN: 1997  
Last Updated on STN: 1997

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Lipase from *Rhizopus oryzae* catalyzes the stereoselective hydrolysis of triglycerides and analogues. Stereopreference and degree of enantiomeric excess of the product varies with the structure of the substrate: trioctanoylglycerol ('ester') and the sn-2 analogues 2-X-1,3-dioctanoylpropandiol, where 2-X = 2-O-octyl ('ether') and 2-hexyl ('alkane'), are preferentially hydrolyzed at sn-1, substitution by 2-phenyl ('phenyl') reverses the stereopreference to sn-3. We have modelled the stereoselectivity of *Rhizopus oryzae* lipase by docking the tetrahedral intermediates of these substrates in two orientations, appropriate to hydrolysis at sn-1 or sn-3, respectively. The initial complexes were further relaxed by molecular dynamics simulations. The favoured orientation of a substrate is characterized by three factors: (1) The substrate fits well into the binding site. The glycerol backbone is relaxed and the sn-2 chain points to a well defined hydrophobic binding site. (2) The oxyanion is stabilized by an extra hydrogen bond from the side chain of Thr 83. (3) The substrate lacks repulsive interactions with protein side chains, especially of Leu 258. Our model is consistent with experimental data and explains qualitatively the ranking of four different substrates with regard to stereoselectivity. It can be used to design lipase mutants with modified stereoselectivity.

L29 ANSWER 24 OF 37 MEDLINE on STN DUPLICATE 7  
ACCESSION NUMBER: 97042947 MEDLINE Full-text  
DOCUMENT NUMBER: PubMed ID: 8888145  
TITLE: The consequences of engineering an extra disulfide bond in the *Penicillium camembertii* mono- and diglyceride specific lipase.  
AUTHOR: Yamaguchi S; Takeuchi K; Mase T; Oikawa K; McMullen T; Derewenda U; McElhaney R N; Kay C M; Derewenda Z S  
CORPORATE SOURCE: Tsukuba Research Laboratories, Amano Pharmaceutical Co. Ltd., Japan.  
SOURCE: Protein engineering, (1996 Sep) Vol. 9, No. 9, pp. 789-95.  
JOURNAL code: 8801484. ISSN: 0269-2139.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199705  
ENTRY DATE: Entered STN: 14 May 1997  
Last Updated on STN: 14 May 1997  
Entered Medline: 6 May 1997

AB The extracellular lipase from *Penicillium camembertii* has unique substrate specificity restricted to mono- and diglycerides. The enzyme is a member of a homologous family of lipases from filamentous fungi. Four of these proteins, from the fungi *Rhizomucor miehei*, *Humicola lanuginosa*, *Rhizopus delemar* and *P. camembertii*, have had their structures elucidated by X-ray crystallography. In spite of pronounced sequence similarities the enzymes exhibit significant differences. For example, the thermostability of the *P. camembertii* lipase is considerably lower than that of the *H. lanuginosa* enzyme. Since only the *P. camembertii* enzyme lacks the characteristic long disulfide bridge, corresponding to Cys22-Cys268 in the *H. lanuginosa* lipase, we have engineered this disulfide into the former enzyme in the hope of obtaining a significantly more stable fold. The properties of the double mutant (Y22C and G269C) were assessed by a variety of biophysical techniques. The extra disulfide link was found to increase the melting temperature of the protein from 51 to 63 degrees C. However, no difference is observed under reducing conditions, indicating an intrinsic instability of the new disulfide. The optimal temperature for catalytic activity decreased by 10 degrees C and the optimum pH was shifted by 0.7 units to more acidic.

L29 ANSWER 25 OF 37 MEDLINE on STN DUPLICATE 8  
ACCESSION NUMBER: 97015915 MEDLINE Full-text  
DOCUMENT NUMBER: PubMed ID: 8862552  
TITLE: The role of Glu87 and Trp89 in the lid of *Humicola lanuginosa* lipase.  
AUTHOR: Martinelle M; Holmquist M; Clausen I G; Patkar S; Svendsen A; Hult K  
CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.  
SOURCE: Protein engineering, (1996 Jun) Vol. 9, No. 6, pp. 519-24.

JOURNAL CODE: 8801484. ISSN: 0269-2139.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199612  
ENTRY DATE: Entered STN: 28 Jan 1997  
Last Updated on STN: 29 Jan 1999  
Entered Medline: 9 Dec 1996

AB The importance of Glu87 and Trp89 in the lid of Humicola lanuginosa lipase for the hydrolytic activity at the water/lipid interface was investigated by site-directed mutagenesis. It was found that the effect on the hydrolytic activity upon the replacement of Trp89 with Phe, Leu, Gly or Glu was substrate dependent. The Trp89 mutants displayed an altered chain length specificity towards triglycerides, with a higher relative activity towards triacetin and trioctanoin compared with tributyrin. Trp89 was shown to be less important in the hydrolysis of vinyl esters compared with ethyl esters and triglycerides. An exclusive effect on the acylation reaction rate by the mutation of Trp89 was consistent with the data. It is suggested that Trp89 is important in the process of binding the acyl chain of the substrate into the active site for optimal acylation reaction rate. The Trp89Phe mutation resulted in an increased hydrolytic activity towards 2-alkylalkanoic acid esters. This is suggested to be due to reduction of unfavourable van der Waals contacts between Trp89 and the 2-substituent of the substrate. Thus, in contrast to natural substrates, Trp89 has a negative impact on the catalytic efficiency when substrates with bulky acyl chains are used. In contrast to the Trp89 mutations, the effect on the hydrolytic activity of the Glu87Ala mutation was almost substrate independent, 35-70% activity of wild-type lipase. A reduction of both the acylation and deacylation reaction was consistent with the data.

L29 ANSWER 26 OF 37 LIFESCI COPYRIGHT 2007 CSA on STN

ACCESSION NUMBER: 1998:29753 LIFESCI Full-text  
TITLE: Acyl glycerol hydrolases: Inhibitors, interface and catalysis  
AUTHOR: Cambillau, C.; Longhi, S.; Nicolas, A.; Martinez, C.  
CORPORATE SOURCE: Architecture et Fonction des Macromolécules Biologiques,  
UPR 9039, CNRS, IFRI, 31 Chemin Joseph Aiguier, 13402  
Marseille, Cedex 20, France  
SOURCE: CURR. OPIN. STRUCT. BIOL., (19960800) vol. 6, no. 4, pp.  
449-455.  
ISSN: 0959-440X.  
DOCUMENT TYPE: Journal  
TREATMENT CODE: General Review  
FILE SEGMENT: K  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB The last five years have witnessed the solution of a large number of lipase structures, which has led, among other insights, to the structural interpretation of the interfacial activation phenomenon in terms of 'lid' opening. This interpretation has been extended this year to include phospholipase A2. Recent structural studies on lipases have provided data on the detailed mechanisms underlying the behaviour of lipases: how they bind to inhibitors or substrates, and what interactions occur between their hydrophobic face and hydrophobic molecules, for example. In addition, studies on cutinase point mutants have shed some light on the role of the oxyanion hole in lipolytic catalysis.

L29 ANSWER 27 OF 37 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1996:113975 BIOSIS Full-text  
DOCUMENT NUMBER: PREV199698686110  
TITLE: Contribution of cutinase serine 42 side chain to the stabilization of the oxyanion transition state.  
AUTHOR(S): Nicolas, Anne; Egmond, Maarten; Verrips, C. Theo; De Vlieg, Jakob; Longhi, Sonia; Cambillau, Christian [Reprint author]; Martinez, Christlaine  
CORPORATE SOURCE: Lab. Cristallographie Cristallisation des Macromolécules Biol., URA1296-CNRS, IFRI, 31 Chemin J. Aiguier, 13402 Marseille cedex 09, France  
SOURCE: Biochemistry, (1996) Vol. 35, No. 2, pp. 398-410.  
CODEN: BICHAW. ISSN: 0006-2960.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 12 Mar 1996  
Last Updated on STN: 10 Jun 1997

AB Cutinase from the fungus Fusarium solani pisi is a lipolytic enzyme able to hydrolyze both aggregated and soluble substrates. It therefore provides a powerful tool for probing the mechanisms underlying lipid hydrolysis. Lipolytic enzymes have a catalytic machinery similar to



those present in serine proteinases. It is characterized by the triad Ser, His, and Asp (Glu) residues, by an oxyanion binding site that stabilizes the transition state via hydrogen bonds with two main chain amide groups, and possibly by other determinants. It has been suggested on the basis of a covalently bound inhibitor that the cutinase oxyanion hole may consist not only of two main chain amide groups but also of the Ser42 O-gamma side chain. Among the esterases and the serine and the cysteine proteases, only *Streptomyces scabies* esterase, subtilisin, and papain, respectively, have a side chain residue which is involved in the oxyanion hole formation. The position of the cutinase Ser42 side chain is structurally conserved in *Rhizomucor miehei* lipase with Ser82 O-gamma, in *Rhizopus delemar* lipase with Thr83 O-gamma-1, and in *Candida antarctica* B lipase with Thr40 O-gamma-1. To evaluate the increase in the tetrahedral intermediate stability provided by Ser42 O-gamma, we mutated Ser42 into Ala. Furthermore, since the proper orientation of Ser42 O-gamma is directed by Asn84, we mutated Asn84 into Ala, Leu, Asp, and Trp, respectively, to investigate the contribution of this indirect interaction to the stabilization of the oxyanion hole. The S42A mutation resulted in a drastic decrease in the activity (450-fold) without significantly perturbing the three-dimensional structure. The N84A and N84L mutations had milder kinetic effects and did not disrupt the structure of the active site, whereas the N84W and N84D mutations abolished the enzymatic activity due to drastic steric and electrostatic effects, respectively.

L29 ANSWER 28 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1996:404098 CAPLUS Full-text  
DOCUMENT NUMBER: 125:133994  
TITLE: A URA3-promoter deletion in a pYES vector increases the expression level of a fungal lipase in *Saccharomyces cerevisiae*  
AUTHOR(S): Okkels, Jens Sigurd  
CORPORATE SOURCE: Novo Nordisk, Copenhagen, DK-2100, Den.  
SOURCE: Annals of the New York Academy of Sciences (1996), 782(Recombinant DNA Biotechnology III), 202-207  
CODEN: ANYAA9; ISSN: 0077-8923  
PUBLISHER: New York Academy of Sciences  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Expression of a lipase gene from *Humicola lanuginosa* in *Saccharomyces cerevisiae* is increased, allowing large-scale purification. A simple deletion of the URA3 promoter from a *S. cerevisiae* expression plasmid was performed. The promoter-deleted plasmid has an increased expression level of fungal lipase gene. The deletion probably causes a poor expression of the URA3 selection marker, probably resulting in a higher copy number per cell of the plasmid. This higher copy number can increase the transcript level per cell and thereby the expression level. In the case of the fungal lipase gene, the expression level with defined inoculum is increased at least three-fold. A part of the 2-µm origin of the pYES type plasmid was also deleted by the URA3 promoter deletion without affecting transformation frequency. The URA3 promoter can easily be deleted from most pYES type plasmids, since most of these plasmids only contain the two PstI sites that remove the whole URA3 promoter.

L29 ANSWER 29 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 9

ACCESSION NUMBER: 1996:490978 CAPLUS Full-text  
DOCUMENT NUMBER: 125:215670  
TITLE: Lipases from *Humicola lanuginosa* adsorbed to hydrophobic surfaces - desorption and activity after addition of surfactants  
AUTHOR(S): Wannerberger, Kristin; Arnebrant, Thomas  
CORPORATE SOURCE: Department of Food Technology, University of Lund, Box 124, Lund, S-22100, Swed.  
SOURCE: Colloids and Surfaces, B: Biointerfaces (1996), 7(3/4), 153-164  
CODEN: CSBBEQ; ISSN: 0927-7765  
PUBLISHER: Elsevier  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The adsorption of lipase to methylated silica and its subsequent removal upon sequential addition of surfactants were measured in situ by ellipsometry. In addition, the specific activity of adsorbed lipase without, and after, exposure to surfactants was determined. Two lipases from *Humicola lanuginosa* were studied, the wild-type (WT) and a mutant which was modified to increase the hydrophobicity of the active-site region. The surfactants used were SDS, C12E5 and sodium dodecanoate and they were all studied in the concentration range  $\leq$  their critical micelle concentration (cmc). In all expts. a non-desorbable fraction remained at the surface. This fraction was larger, however, for the WT compared to the mutant. The concentration, expressed as a fraction of the cmc, of surfactant inducing the most pronounced desorption was found to vary among the surfactants. Desorption occurred at increasing fractions of the cmc in the order C12E5, sodium dodecanoate and SDS. This was found for both the WT and the mutant. No significant effect of C12E5 or SDS on the specific activities could be observed while the exposure to sodium dodecanoate markedly increased the specific activity of both lipases.

L29 ANSWER 30 OF 37 MEDLINE on STN DUPLICATE 10

ACCESSION NUMBER: 95242058 MEDLINE Full-text  
DOCUMENT NUMBER: PubMed ID: 7536956  
TITLE: The importance of non-charged amino acids in antibody binding to *Humicola lanuginosa* lipase.  
AUTHOR: Naver H; Lovborg U  
CORPORATE SOURCE: Novo Nordisk Industrial Biotechnology, Copenhagen, Denmark.  
SOURCE: Scandinavian journal of immunology, (1995 May) Vol. 41, No. 5, pp. 443-8.  
Journal code: 0323767. ISSN: 0300-9475.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199505  
ENTRY DATE: Entered STN: 5 Jun 1995  
Last Updated on STN: 29 Jan 1999  
Entered Medline: 25 May 1995

AB The antigenicity of 36 *Humicola lanuginosa* lipase (HL) variants, generated by site directed mutagenesis, was compared with that of the unchanged enzyme. Polyclonal antibodies raised against variant lipases were investigated and compared with the antibodies raised against the wild type lipase in an ELISA competition assay. The results showed that exchange of charged amino acids with polar residues in surface epitopes of HL, results in a tighter binding of the antibody to the epitope. Four amino acids (Trp at position 89, Asp at positions 96 and 254 and Phe at position 211) were found to be essential for antibody binding in each their epitope of the wild type enzyme.

L29 ANSWER 31 OF 37 MEDLINE on STN DUPLICATE 11

ACCESSION NUMBER: 95391076 MEDLINE Full-text  
DOCUMENT NUMBER: PubMed ID: 7662109  
TITLE: Probing a functional role of Glu87 and Trp89 in the lid of *Humicola lanuginosa* lipase through transesterification reactions in organic solvent.  
AUTHOR: Holmquist M; Clausen I G; Patkar S; Svendsen A; Hult K  
CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.  
SOURCE: Journal of protein chemistry, (1995 May) Vol. 14, No. 4, pp. 217-24.  
Journal code: 8217321. ISSN: 0277-8033.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199510  
ENTRY DATE: Entered STN: 20 Oct 1995  
Last Updated on STN: 29 Jan 1999  
Entered Medline: 10 Oct 1995

AB To reveal the functional role of Glu87 and Trp89 in the lid of *Humicola lanuginosa* lipase, site-directed mutagenesis at Glu87 and Trp89 was carried out. The catalytic performance of wild-type and mutated lipases was studied in transesterification reactions in cyclohexane at a controlled water activity. Two different acyl donors were used in the investigation: tributyrin, a natural substrate for a lipase, and vinyl butyrate, an activated ester suitable for fast and efficient lipase-catalyzed transformations in preparative organic synthesis. As acyl acceptor 1-heptanol was used. The Glu87Ala mutation decreased the  $V_{max,app}$  value with tributyrin and vinyl butyrate by a factor of 1.5 and 2, respectively. The  $K_{m,app}$  for tributyrin was not affected by the Glu87Ala mutation, but the  $K_{m,app}$  for vinyl butyrate increased twofold compared to the wild-type lipase. Changing Trp89 into a Phe residue afforded an enzyme with a 2.7- and 2-fold decreased  $V_{max,app}$  with the substrates tributyrin and vinyl butyrate, respectively, compared to the wild-type lipase. No significant effects on the  $K_{m,app}$  values for tributyrin or vinyl butyrate were seen as a result of the Trp89Phe mutation. However, the introduction of a Glu residue at position 89 in the lid increased the  $K_{m,app}$  for tributyrin and vinyl butyrate by a factor of > 5 and 2, respectively. The Trp89Glu mutated lipase could not be saturated with tributyrin within the experimental conditions (0-680 mM) studied here. With vinyl butyrate as a substrate the  $V_{max,app}$  was only 6% of that obtained with wild-type enzyme.

L29 ANSWER 32 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1995:340822 CAPLUS Full-text  
DOCUMENT NUMBER: 122:155213  
TITLE: Lipase variants, their manufacture with recombinant cells, and their use in detergent

INVENTOR(S): compositions  
Svendsen, Allan; Patkar, Shamkant Anant; Gormsen,  
Erik; Clausen, Ib Groth  
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.  
SOURCE: PCT Int. Appl., 44 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 5  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9425577	A1	19941110	WO 1994-DK162	19940422
W: AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KG, KP, KR, KZ, LK, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, TJ, UA, US, UZ, VN				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9465358	A	19941121	AU 1994-65358	19940422
BR 9406384	A	19960116	BR 1994-6384	19940422
EP 695348	A1	19960207	EP 1994-913051	19940422
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
CN 1124039	A	19960605	CN 1994-192175	19940422
JP 08509364	T	19961008	JP 1994-523626	19940422
FI 9505018	A	19951020	FI 1995-5018	19951020
PRIORITY APPLN. INFO.:				
			DK 1993-466	A 19930423
			WO 1994-DK162	W 19940422

AB Novel lipase variants with improved properties, DNA constructs coding for the expression of these. Variants, host cells expressing the lipase variant genes, and methods for preparing the variants by culturing the recombinant cells are claimed. Lipases comprising a trypsin-like catalytic triad including an active serine located in a predominantly hydrophobic, elongated binding pocket of the lipase mol. are mutated so as to substitute a non-aromatic amino acid residue of a lipid contact zone comprising residues located within the part of the lipase structure containing the active serine residue, which residues may participate in the interaction with the substrate at or during hydrolysis, with an aromatic amino acid residue. Several *Humicola lanuginosa* lipase variants were produced with *Aspergillus oryzae* and tested for wash performance. The [Trp-96]-, and [Trp-96,Asn-210]- lipase variants exhibited 2.7 and 3.4-fold better wash performance than wild-type lipase.

L29 ANSWER 33 OF 37 MEDLINE on STN DUPLICATE 12

ACCESSION NUMBER: 95115502 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 7815893

TITLE: Trp89 in the lid of *Humicola lanuginosa*  
lipase is important for efficient hydrolysis of tributyrin.

AUTHOR: Holmquist M; Martinelle M; Clausen I G; Patkar S; Svendsen A; Hult K

CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.

SOURCE: Lipids, (1994 Sep) Vol. 29, No. 9, pp. 599-603.  
Journal code: 0060450. ISSN: 0024-4201.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199502

ENTRY DATE: Entered STN: 17 Feb 1995  
Last Updated on STN: 29 Jan 1999  
Entered Medline: 9 Feb 1995

AB To determine whether Trp89 located in the lid of the lipase (EC 3.1.1.3) from *Humicola lanuginosa* is important for the catalytic property of the enzyme, site-directed mutagenesis at Trp89 was carried out. The kinetic properties of wild type and mutated enzymes were studied with tributyrin as substrate. Lipase variants in which Trp89 was changed to Phe, Leu, Gly or Glu all showed less than 14% of the activity compared to that of the wild type lipase. The Trp89Glu mutant was the least active with only 1% of the activity seen with the wild type enzyme. All Trp mutants had the same binding affinity to the tributyrin substrate interface as did the wild type enzyme. Wild type lipase showed saturation kinetics against tributyrin when activities were measured with mixed emulsions containing different proportions of tributyrin and the nonionic alkyl polyoxyethylene ether surfactant, Triton DF-16. Wild type enzyme showed a  $V_{max} = 6000 \pm 300$  mmol.min<sup>-1</sup>.g<sup>-1</sup> and an apparent  $K_m = 16 \pm 2\%$  (vol/vol) for tributyrin in Triton DF-16, while the mutants did not show saturation kinetics in an identical assay. The apparent  $K_m$  for tributyrin in Triton DF-16 was increased as the result of replacing Trp89 with other residues (Phe, Leu, Gly or Glu). The activities of all mutants were more sensitive to the presence of Triton DF-16 in the tributyrin substrate than was wild type lipase. The activity of the Trp89Glu mutant was decreased to 50% in

the presence of 2 vol% Triton DF-16 compared to the activity seen with pure tributyrin as substrate. (ABSTRACT TRUNCATED AT 250 WORDS)

L29 ANSWER 34 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1995:18786 CAPLUS Full-text  
DOCUMENT NUMBER: 122:34002  
TITLE: Detergent compositions comprising lipase variants  
AUTHOR(S): Novo, Nordisk  
CORPORATE SOURCE: Den.  
SOURCE: Research Disclosure (1994), 359, 151-6 (No. 35944)  
CODEN: RSDSBB; ISSN: 0374-4353  
DOCUMENT TYPE: Journal; Patent  
LANGUAGE: English  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
-----	---	-----	-----	-----
RD 359044		19940310		

PRIORITY APPLN. INFO.: RD 1994-359044 19940310

AB Detergent comps., especially for dishwashing, contain lipase from *Humicola lanuginosa* for improved effectiveness.

L29 ANSWER 35 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1993:576669 CAPLUS Full-text  
DOCUMENT NUMBER: 119:176669  
TITLE: Improving the resistance of proteins and enzymes to proteolysis by manipulation of surface structures  
INVENTOR(S): Svendsen, Allan; Clausen, Ib Groth; Pathar, Shamkant Anant  
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.  
SOURCE: PCT Int. Appl., 55 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
-----	---	-----	-----	-----
WO 9311254	A1	19930610	WO 1992-DK351	19921126
W: BR, CA, FI, JP, KR, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
JP 07504807	T	19950601	JP 1992-509724	19921126
EP 667915	A1	19950823	EP 1992-924587	19921126
R: AT, BE, DE, DK, ES, FR, GB, IT, NL				
BR 9206815	A	19951031	BR 1992-6815	19921126
FI 9402467	A	19940526	FI 1994-2467	19940526

PRIORITY APPLN. INFO.: WO 1991-DK350 A 19911126  
DK 1950-91003 A 19911126  
DK 1950-92003 A 19911126  
WO 1992-DK351 W 19921126

AB Enzymes or other proteins are made more resistant to proteinases by modification of the sequences of surface loops that are common sites of proteolysis. The method is particularly suited for enzymes to be used in proteinase-containing laundry detergents. These sites are identified by very limited proteolysis of the protein and N-terminal sequencing of the cleavage products. Three cleavage sites identified the lipase of *Humicola lanuginosa* DSM 4106 at positions 162-165, 209-212, and 239-242 were substituted with an array of tetrapeptides by standard methods of site-directed mutagenesis of the cloned gene and the proteins manufactured by expression of the gene in *Aspergillus oryzae*. Under conditions in which the wild-type proteinase lost 75% of its activity in 90 min upon digestion with Savinase® (subtilisin), one of these variants retained >80% of its activity.

L29 ANSWER 36 OF 37 MEDLINE on STN DUPLICATE 13

ACCESSION NUMBER: 94183411 MEDLINE Full-text  
DOCUMENT NUMBER: PubMed ID: 8136025  
TITLE: Lipases from *Rhizomucor miehei* and *Humicola lanuginosa*: modification of the lid covering the active site alters enantioselectivity.  
AUTHOR: Holmquist M; Martinelle M; Berglund P; Clausen I G; Patkar S; Svendsen A; Hult K  
CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal

SOURCE: Institute of Technology, Stockholm, Sweden.  
Journal of protein chemistry, (1993 Dec) Vol. 12, No. 6,  
pp. 749-57.  
Journal code: 8217321. ISSN: 0277-8033.

PUB. COUNTRY: United States

DOCUMENT TYPE: (COMPARATIVE STUDY)  
Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199404

ENTRY DATE: Entered STN: 9 May 1994  
Last Updated on STN: 29 Jan 1999  
Entered Medline: 28 Apr 1994

AB The homologous lipases from *Rhizomucor miehei* and *Humicola lanuginosa* showed approximately the same enantioselectivity when 2-methyldecanoic acid esters were used as substrates. Both lipases preferentially hydrolyzed the S-enantiomer of 1-heptyl 2-methyldecanoate (*R. miehei*: ES = 8.5; *H. lanuginosa*: ES = 10.5), but the R-enantiomer of phenyl 2-methyldecanoate (ER = 2.9). Chemical arginine specific modification of the *R. miehei* lipase with 1,2-cyclohexanedione resulted in a decreased enantioselectivity (ER = 2.0), only when the phenyl ester was used as a substrate. In contrast, treatment with phenylglyoxal showed a decreased enantioselectivity (ES = 2.5) only when the heptyl ester was used as a substrate. The presence of guanidine, an arginine side chain analog, decreased the enantioselectivity with the heptyl ester (ES = 1.9) and increased the enantioselectivity with the aromatic ester (ER = 4.4) as substrates. The mutation, Glu 87 Ala, in the lid of the *H. lanuginosa* lipase, which might decrease the electrostatic stabilization of the open-lid conformation of the lipase, resulted in 47% activity compared to the native lipase, in a tributyrin assay. The Glu 87 Ala mutant showed an increased enantioselectivity with the heptyl ester (ES = 17.4) and a decreased enantioselectivity with the phenyl ester (ER = 2.5) as substrates, compared to native lipase. The enantioselectivities of both lipases in the esterification of 2-methyldecanoic acid with 1-heptanol were unaffected by the lid modifications.

L29 ANSWER 37 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1993:142540 CAPLUS Full-text

DOCUMENT NUMBER: 118:142540

TITLE: Stabilization of enzymes by selective replacement of amino acids with proline

INVENTOR(S): Svendsen, Allan; Von der Osten, Claus; Clausen, Ib  
Groth; Patkar, Shamkant Anant; Borch, Kim

PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.

SOURCE: PCT Int. Appl., 45 pp.  
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9219726	A1	19921112	WO 1992-DK142	19920501
W: FI, JP, KR, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE				
EP 585285	A1	19940309	EP 1992-910123	19920501
EP 585285	B1	19980812		
R: AT, BE, DE, DK, ES, FR, GB, IT, NL, SE				
JP 06507071	T	19940811	JP 1992-509154	19920501
AT 169678	T	19980815	AT 1992-910123	19920501
ES 2121854	T3	19981216	ES 1992-910123	19920501
US 5914306	A	19990622	US 1993-140008	19931022
PRIORITY APPLN. INFO.:				
			EP 1991-610035	A 19910501
			WO 1992-DK142	W 19920501

AB Enzymes are made more stable by methods that minimize the structural flexibility of the peptide backbone. This is achieved by substituting selected amino acids with proline in regions of the protein outside stable higher order structures such as  $\alpha$ -helices and  $\beta$ -sheets. The criteria for selecting amino acids to be substituted also include dihedral angles  $\psi$  and  $\phi$  in the ranges -90° <  $\phi$  < -40° and -180° <  $\psi$  < -150°, -80° <  $\psi$  < 10°, 100° <  $\psi$  < 180°. The invention is intended for use in the preparation of stable enzymes for use in detergents. Genes for a set of derivs. of the lipase of *Humicola lanuginosa* with individual amino acids substituted by proline were prepared by PCR mutagenesis of the gene and the expression cassette used to manufacture the protein in *Aspergillus*. An analog of the lipase with Gly225 replaced by Pro lost 50% of its activity after .apprx.30 min at 70° whereas the wild type lost 50% of its activity after 15 min. at the same temperature

## STN Search

10/779,427

FILE 'HOME' ENTERED AT 13:45:34 ON 29 MAY 2007

=&gt; file .nash

=&gt; s (lipolytic enzyme or lipase) and lanuginosa and (muta? or variant) and 263

L1 0 FILE MEDLINE  
L2 0 FILE CAPLUS  
L3 0 FILE SCISEARCH  
L4 0 FILE LIFESCI  
L5 0 FILE BIOSIS  
L6 0 FILE EMBASE

TOTAL FOR ALL FILES

L7 0 (LIPOLYTIC ENZYME OR LIPASE) AND LANUGINOSA AND (MUTA? OR VARIAN  
T) AND 263

=&gt; s (lipolytic enzyme or lipase) and lanuginosa and (muta? or variant)

TOTAL FOR ALL FILES

L14 230 (LIPOLYTIC ENZYME OR LIPASE) AND LANUGINOSA AND (MUTA? OR VARIAN  
T)

=&gt; s (lipolytic enzyme or lipase) and lanuginosa and 263

TOTAL FOR ALL FILES

L21 0 (LIPOLYTIC ENZYME OR LIPASE) AND LANUGINOSA AND 263

=&gt; s (lipolytic enzyme or lipase) and lanuginosa and 4109

TOTAL FOR ALL FILES

L28 7 (LIPOLYTIC ENZYME OR LIPASE) AND LANUGINOSA AND 4109

=&gt; s l14 not 2000-2007/py

TOTAL FOR ALL FILES

L49 85 L14 NOT 2000-2007/PY

=&gt; dup rem l49

PROCESSING COMPLETED FOR L49

L50 37 DUP REM L49 (48 DUPLICATES REMOVED)

=&gt; d ibib abs 1-37

L50 ANSWER 1 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1999:231214 CAPLUS Full-text

DOCUMENT NUMBER: 130:264043

TITLE: Lipase variants and their use in  
detergent compositionsINVENTOR(S): Svendsen, Allan; Patkar, Shamkant Anant; Gormsen,  
Erik; Clausen, Ib Groth; Okkels, Jens Sigurd;  
Thellersen, Marianne

PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.

SOURCE: U.S., 50 pp., Cont.-in-part of U.S. Ser. No. 434,904,  
abandoned.

CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 5

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5892013	A	19990406	US 1995-488271	19950905
CA 2092615	A1	19920314	CA 1991-2092615	19910913
PRIORITY APPLN. INFO.:			DK 1990-2194	A 19900913
			DK 1990-2195	A 19900913
			DK 1990-2196	A 19900913
			US 1993-977429	B1 19930222
			DK 1993-466	A 19930423
			DK 1994-217	A 19940222
			US 1995-434904	B2 19950501

AB The present invention relates to lipase variants which exhibit improved properties, detergent compns. comprising said lipase variants, DNA constructs coding for said lipase variants, and methods of making said lipase variants. The variants contain one or more deletions or substitutions of an amino acid residue in the lipid contact zone, or substitutions of two amino

acid residues of the surface loop structure with cysteine which are positioned to form a disulfide bond. Numerous *Humicola lanuginosa* lipase variants were prepared with recombinant *Aspergillus oryzae*. Variants R209A+E210A, E56Q, D96L, D96K, D96W, and D96W+E210N had considerably better wash performance than the wild-type lipase. A number of the variants had higher specific activity and/or improved resistance to proteolytic degradation and almost all had improved resistance to alkaline conditions.

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L50 ANSWER 2 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN  
 ACCESSION NUMBER: 1999:111576 CAPLUS Full-text  
 DOCUMENT NUMBER: 130:164901  
 TITLE: Lipase variants and their use in detergent compositions  
 INVENTOR(S): Svendsen, Allan; Patkar, Shamkant Anant; Gormsen, Erik; Okkels, Jens Sigurd; Thellersen, Marianne  
 PATENT ASSIGNEE(S): Novo Nordisk A/S, Norway  
 SOURCE: U.S., 51 pp., Cont.-in-part of U.S. Ser. No. 434,904, abandoned.  
 CODEN: USXXAM  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 5  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5869438	A	19990209	US 1995-479275	19950607
CA 2092615	A1	19920314	CA 1991-2092615	19910913
PRIORITY APPLN. INFO.:			DK 1990-2194	A 19900913
			DK 1990-2195	A 19900913
			DK 1990-2196	A 19900913
			US 1991-977429	B1 19910913
			DK 1993-466	A 19930423
			DK 1994-217	A 19940222
			US 1995-434904	B2 19950501

AB The present invention relates to lipase variants which exhibit improved properties and to detergent additives and detergent compns. comprising said lipase variants. The variants contain one or more deletions or substitutions of an amino acid residue in the lipid contact zone, or substitutions of two amino acid residues of the surface loop structure with cysteine which are positioned to form a disulfide bond. Numerous *Humicola lanuginosa* lipase variants were prepared with recombinant *Aspergillus oryzae*. Variants R209A+E210A, E56Q, D96L, D96K, D96W, and D96W+E210N had considerably better wash performance than the wild-type lipase. A number of the variants had higher specific activity and/or improved resistance to proteolytic degradation and almost all had improved resistance to alkaline conditions.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L50 ANSWER 3 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN  
 ACCESSION NUMBER: 1999:785630 SCISEARCH Full-text  
 THE GENUINE ARTICLE: 246MN  
 TITLE: Computational analysis of chain flexibility and fluctuations in *Rhizomucor miehei* lipase  
 AUTHOR: Peters G H; Bywater R P (Reprint)  
 CORPORATE SOURCE: Novo Nordisk AS, Biostruct Grp, DK-2760 Malov, Denmark (Reprint); Tech Univ Denmark, Dept Chem, DK-2800 Lyngby, Denmark  
 COUNTRY OF AUTHOR: Denmark  
 SOURCE: PROTEIN ENGINEERING, (SEP 1999) Vol. 12, No. 9, pp. 747-754.  
 ISSN: 0269-2139.  
 PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.  
 DOCUMENT TYPE: Article; Journal  
 LANGUAGE: English  
 REFERENCE COUNT: 77  
 ENTRY DATE: Entered STN: 1999  
 Last Updated on STN: 1999

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB We have performed molecular dynamics simulation of *Rhizomucor miehei* lipase (Rml) with explicit water molecules present. The simulation was carried out in periodic boundary conditions and conducted for 1.2 ns in order to determine the concerted protein dynamics and to examine how well the essential motions are preserved along the trajectory. Protein motions are extracted by means of the essential dynamics analysis method for different

lengths of the trajectory. Motions described by eigenvector 1 converge after approximately 200 ps and only small changes are observed with increasing simulation time. Protein dynamics along eigenvectors with larger indices, however, change with simulation time and generally, with increasing eigenvector index, longer simulation times are required for observing similar protein motions (along a particular eigenvector). Several regions in the protein show relatively large fluctuations and in particular motions in the active site lid and the segments Thr57-Asn63 and the active site hinge region Pro101-Gly104 are seen along several eigenvectors. These motions are generally associated with glycine residues, while no direct correlations are observed between these fluctuations and the positioning of prolines in the protein structure. The partial opening/closing of the lid is an example of induced fit mechanisms seen in other enzymes and could be a general mechanism for the activation of Rml.

L50 ANSWER 4 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:50858 SCISEARCH [Full-text](#)  
 THE GENUINE ARTICLE: 155QB  
 TITLE: Stereoselectivity of Mucorales lipases toward triacylglycerols - A simple solution to a complex problem  
 AUTHOR: Scheib H; Pleiss J; Kovac A; Paltauf F; Schmid R D (Reprint)  
 CORPORATE SOURCE: Univ Stuttgart, Inst Tech Biochem, Allmandring 31, D-70569 Stuttgart, Germany (Reprint); Univ Stuttgart, Inst Tech Biochem, D-70569 Stuttgart, Germany; Graz Tech Univ, Dept Biochem & Food Chem, A-8010 Graz, Austria  
 COUNTRY OF AUTHOR: Germany; Austria  
 SOURCE: PROTEIN SCIENCE, (JAN 1999) Vol. 8, No. 1, pp. 215-221. ISSN: 0961-8368.  
 PUBLISHER: COLD SPRING HARBOR LAB PRESS, 1 BUNGTOWN RD, PLAINVIEW, NY 11724 USA.  
 DOCUMENT TYPE: Article; Journal  
 LANGUAGE: English  
 REFERENCE COUNT: 21  
 ENTRY DATE: Entered STN: 1999  
 Last Updated on STN: 1999

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The lipases from Rhizopus and Rhizomucor are members of the family of Mucorales lipases. Although they display high sequence homology, their stereoselectivity toward triacylglycerols (sn-2 substituted triacylglycerols) varies. Four different triacylglycerols were investigated, which were classified into two groups: flexible substrates with rotatable O'-C1' ether or ester bonds adjacent to C2 of glycerol and rigid substrates with a rigid N'-C1' amide bond or a phenyl ring in sn-2. Although Rhizopus lipase shows opposite stereopreference for flexible and rigid substrates (hydrolysis in sn-1 and sn-3, respectively), Rhizomucor lipase hydrolyzes both groups of triacylglycerols preferably in sn-1. To explain these experimental observations, computer-aided molecular modeling was applied to study the molecular basis of stereoselectivity. A generalized model for both lipases of the Mucorales family highlights the residues mediating stereoselectivity: (1) L258, the C-terminal neighbor of the catalytic histidine, and (2) G266, which is located in a loop contacting the glycerol backbone of a bound substrate. Interactions with triacylglycerol substrates are dominated by van der Waals contacts. Stereoselectivity can be predicted by analyzing the value of a single substrate torsion angle that discriminates between sn-1 and sn-3 stereopreference for all substrates and lipases investigated here. This simple model can be easily applied in enzyme and substrate engineering to predict Mucorales lipase variants and synthetic substrates with desired stereoselectivity.

L50 ANSWER 5 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1999:287580 CAPLUS [Full-text](#)  
 DOCUMENT NUMBER: 131:84811  
 TITLE: Steady state fluorescence studies on the interaction of Humicola lanuginosa lipase with lipid vesicles  
 AUTHOR(S): Cajal, Y.; Prat, J.; Svendsen, A.; De Bolos, J.; Alsina, M. A.  
 CORPORATE SOURCE: Department of Physical Chemistry, School of Pharmacy, University of Barcelona, Barcelona, 08028, Spain  
 SOURCE: Biomedical Chromatography (1999), 13(2), 157-158 CODEN: BICHE2; ISSN: 0269-3879  
 PUBLISHER: John Wiley & Sons Ltd.  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB In this work, the conformational changes that take place in Humicola lanuginosa lipase (HLL) upon binding to vesicles of 1-palmitoyl-2-oleoylglycerol-sn-phosphoglycerol (POPG), and also in the



presence of tributyrin (TB) as substrate, were studied by fluorescence techniques. The catalytic activity of the enzyme under the same conditions as used for fluorescence was measured spectroscopically by UV using p-nitrophenylbutyrate as substrate. An inactive mutant, with active site Ser-146 mutated to Ala, was used in the fluorescence expts. to avoid interference from product formation.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L50 ANSWER 6 OF 37 MEDLINE on STN DUPLICATE 1  
 ACCESSION NUMBER: 1998400942 MEDLINE Full-text  
 DOCUMENT NUMBER: PubMed ID: 9730809  
 TITLE: Active serine involved in the stabilization of the active site loop in the *Humicola lanuginosa* lipase.  
 AUTHOR: Peter's G H; Svendsen A; Langberg H; Vind J; Patkar S A; Toxvaerd S; Kinnunen P K  
 CORPORATE SOURCE: Chemistry Department III, H.C. Orsted Institutet, University of Copenhagen, Denmark.. ghp@st.ki.ku.dk  
 SOURCE: Biochemistry, (1998 Sep 8) Vol. 37, No. 36, pp. 12375-83. Journal code: 0370623. ISSN: 0006-2960.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199902  
 ENTRY DATE: Entered STN: 23 Feb 1999  
 Last Updated on STN: 23 Feb 1999  
 Entered Medline: 5 Feb 1999

AB We have investigated the binding properties of and dynamics in *Humicola lanuginosa* lipase (H11) and the inactive mutant S146A (active Ser146 substituted with Ala) using fluorescence spectroscopy and molecular dynamics simulations, respectively. H11 and S146A show significantly different binding behavior for phosphatidylcholine (PC) and phosphatidylglycerol (PG) liposomes. Generally, higher binding affinity is observed for H11 than the S146A mutant. Furthermore, depending on the matrix, the addition of the transition state analogue benzene boronic acid increases the binding affinity of S146A, whereas only small changes are observed for H11 suggesting that the active site lid in the latter opens more easily and hence more lipase molecules are bound to the liposomes. These observations are in agreement with molecular dynamics simulations and subsequent essential dynamics analyses. The results reveal that the hinges of the active site lid are more flexible in the wild-type H11 than in S146A. In contrast, larger fluctuations are observed in the middle region of the active site loop in S146A than in H11. These findings reveal that the single mutation (S146A) of the active site serine leads to substantial conformational alterations in the *H. lanuginosa* lipase and different binding affinities.

L50 ANSWER 7 OF 37 MEDLINE on STN DUPLICATE 2  
 ACCESSION NUMBER: 1998244839 MEDLINE Full-text  
 DOCUMENT NUMBER: PubMed ID: 9578545  
 TITLE: Interfacial activation of triglyceride lipase from *Thermomyces* (*Humicola*) *lanuginosa*: kinetic parameters and a basis for control of the lid.  
 AUTHOR: Berg O G; Cajal Y; Butterfoss G L; Grey R L; Alsina M A; Yu B Z; Jain M K  
 CORPORATE SOURCE: Department of Chemistry and Biochemistry, University of Delaware, Newark 19716, USA.  
 CONTRACT NUMBER: GM29703 (NIGMS)  
 SOURCE: Biochemistry, (1998 May 12) Vol. 37, No. 19, pp. 6615-27. Journal code: 0370623. ISSN: 0006-2960.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: (COMPARATIVE STUDY) Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T) (RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199806  
 ENTRY DATE: Entered STN: 11 Jun 1998  
 Last Updated on STN: 29 Jan 1999  
 Entered Medline: 4 Jun 1998

AB A strategy is developed to analyze steady-state kinetics for the hydrolysis of a soluble substrate partitioned into the interface by an enzyme at the interface. The feasibility of this approach to obtain interfacial primary kinetic and equilibrium parameters is demonstrated for a triglyceride lipase. Analysis for phospholipase A2 catalyzed hydrolysis of rapidly exchanging micellar (Berg et al. (1997) Biochemistry 36, 14512-14530) and nonexchangeable vesicular (Berg et al., (1991) Biochemistry 30, 7283-7291) phospholipids is extended to include the case of a substrate that does

not form the interface. The triglyceride lipase (t1TGL) from *Thermomyces* (formerly *Humicola*) *lanuginosa* hydrolyzes p-nitrophenylbutyrate or tributyrin partitioned in the interface of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) vesicles at a rate that is more than 100-fold higher than that for the monodispersed substrate or for the substrate partitioned into zwitterionic vesicles. Catalysis and activation is not seen with the S146A mutant without the catalytic serine-146; however, it binds to the POPG interface with the same affinity as the WT. Thus POPG acts as a diluent surface to which the lipase binds in an active, or "open", form for the catalytic turnover; however, the diluent molecules have poor affinity for the active site. Analysis of the substrate and the diluent concentration dependence of the rate of hydrolysis provides a basis for the determination of the primary interfacial catalytic parameters. As a competitive substrate, tributyrin provided a check for the apparent affinity parameters. Nonidealities from the fractional difference in the molecular areas in interfaces are expressed as the area correction factor and can be interpreted as a first-order approximation for the interfacial activity coefficient. The basis for the interfacial activation of t1TGL on anionic interface is attributed to cationic R81, R84, and K98 in the "hinge" around the 86-93 "lid" segment of t1TGL.

L50 ANSWER 8 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 3

ACCESSION NUMBER: 1998:711158 CAPLUS Full-text  
 DOCUMENT NUMBER: 130:92064  
 TITLE: Spectroscopic studies of the interfacial binding of  
*Humicola lanuginosa* lipase  
 AUTHOR(S): Cajal, Yolanda; Prat, Josefina; De Bolos, Jordi;  
 Asuncion Alsina, M.; Svendsen, Allan  
 CORPORATE SOURCE: School of Pharmacy, Department of Physical Chemistry,  
 University of Barcelona, Barcelona, 08028, Spain  
 SOURCE: Analyst (Cambridge, United Kingdom) (1998), 123(11),  
 2229-2233  
 CODEN: ANALAO; ISSN: 0003-2654  
 PUBLISHER: Royal Society of Chemistry  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB The interaction of *Humicola lanuginosa* lipase (HLL) with small unilamellar vesicles of 1-palmitoyl-2-oleoylglycero-sn-3-phosphoglycerol (POPG) and in the presence of tributyrin (TB) as a substrate was studied by the use of steady-state fluorescence techniques. An inactive mutant with the serine from the catalytic triad changed by alanine (S146A) was used in expts. with TB to avoid interferences from product formation. HLL binds to POPG vesicles in an active or open form for the catalytic turnover, therefore POPG provides a suitable system for studying the conformational changes involving the movement of the loop of amino acids that covers the active site of the enzyme in solution. Tryptophan (Trp) fluorescence shows that HLL binding to POPG occurs with a change in the environment of Trp residue(s) and that there is only one type of bound form, even in the presence of TB. Accessibility to aqueous quenchers indicates shielding of Trp in the membrane. Fluorescence anisotropy of the enzyme increases on binding to the vesicles, indicating restricted rotational freedom for the Trp due to penetration in the bilayer. Resonance energy transfer expts. using an interfacial membrane probe, 1-[4-(trimethylammonio)phenyl]-6-phenylhexa-1,3,5-triene p-toluenesulfonate (TMA-DPH), and an internal membrane probe, 1,6-diphenylhexa-1,3,5-triene (DPH), indicate that HLL does not penetrate very deeply in the hydrophobic core of the membrane, but preferentially stays close to the lipid interface. Addition of substrate (TB) does not result in any addnl. changes in the spectroscopic properties of HLL. It is suggested that the observed changes are due to the 'opening of the lid' on binding to POPG vesicles, leaving the active site accessible for the substrate to bind.

REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS  
 RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L50 ANSWER 9 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on  
 STN

ACCESSION NUMBER: 1998:229525 SCISEARCH Full-text  
 THE GENUINE ARTICLE: ZC005  
 TITLE: A proposed architecture for lecithin cholesterol acyl  
 transferase (LCAT): Identification of the catalytic triad  
 and molecular modeling  
 AUTHOR: Peelman F; Vinaimont N; Verhee A; Vanloo B; Verschelde J  
 L; Labeur C; Seguret-Mace S; Duverger N; Hutchinson G;  
 Vandekerckhove J; Tavernier J; Rosseneu M (Reprint)  
 CORPORATE SOURCE: Univ Ghent, Dept Biochem, Lab Lipoprot Chem, Hosp Str 13,  
 B-9000 Ghent, Belgium (Reprint); Univ Ghent, Dept Biochem,  
 Lab Lipoprot Chem, B-9000 Ghent, Belgium; State Univ Ghent  
 VIB, Fac Med, Dept Biochem, B-9000 Ghent, Belgium; Rhone  
 Poulenc Rorer, GENCELL, Cardiovasc Dept, Vitry Sur Seine,  
 France; Univ Coll London, Dept Biochem, London, England  
 maryvonne.rosseneu@rug.ac.be  
 COUNTRY OF AUTHOR: Belgium; France; England  
 SOURCE: PROTEIN SCIENCE, (MAR 1998) Vol. 7, No. 3, pp. 587-599.  
 ISSN: 0961-8368.

PUBLISHER: COLD SPRING HARBOR LAB PRESS, PUBLICATIONS DEPT, 500  
SUNNYSIDE BLVD, WOODBURY, NY 11797-2924 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 52  
ENTRY DATE: Entered STN: 1998  
Last Updated on STN: 15 Mar 2007

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The enzyme cholesterol lecithin acyl transferase (LCAT) shares the Ser/Asp-Glu/His triad with lipases, esterases and proteases, but the low level of sequence homology between LCAT and these enzymes did not allow for the LCAT fold to be identified yet. We, therefore, relied upon structural homology calculations using threading methods based on alignment of the sequence against a library of solved three-dimensional protein structures, for prediction of the LCAT fold. We propose that LCAT, like lipases, belongs to the alpha/beta hydrolase fold family, and that the central domain of LCAT consists of seven conserved parallel beta-strands connected by four alpha-helices and separated by loops. We used the conserved features of this protein fold for the prediction of functional domains in LCAT, and carried out site-directed mutagenesis for the localization of the active site residues. The wild-type enzyme and mutants were expressed in Cos-1 cells. LCAT mass was measured by ELISA, and enzymatic activity was measured on recombinant HDL, on LDL and on a monomeric substrate. We identified D345 and H377 as the catalytic residues of LCAT, together with F103 and L182 as the oxyanion hole residues. In analogy with lipases, we further propose that a potential "lid" domain at residues 50-74 of LCAT might be involved in the enzyme-substrate interaction. Molecular modeling of human LCAT was carried out using human pancreatic and *Candida antarctica* lipases as templates. The three-dimensional model proposed here is compatible with the position of natural mutants for either LCAT deficiency or Fish-eye disease. It enables moreover prediction of the LCAT domains involved in the interaction with the phospholipid and cholesterol substrates.

L50 ANSWER 10 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 4

ACCESSION NUMBER: 1998:811251 CAPLUS Full-text

DOCUMENT NUMBER: 130:164804

TITLE: Fluorescence study of fungal lipase from  
*Humicola lanuginosa*

AUTHOR(S): Stobiecka, A.; Wysocki, S.; Brzozowski, A. M.

CORPORATE SOURCE: Inst. General Food Chem., Tech. Univ., Lodz, 90-924,  
Pol.

SOURCE: Journal of Photochemistry and Photobiology, B: Biology  
(1998), 45(2-3), 95-102

CODEN: JPPBEG; ISSN: 1011-1344

PUBLISHER: Elsevier Science S.A.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Time-resolved and steady-state fluorescence quenching measurements have been performed to study two different conformations of the fungal lipase from *Humicola lanuginosa*. The intrinsic fluorescence of tryptophan Trp89 residue, located in the 'lid' region, has been used as a probe for the dynamics of protein. The native ('closed-lid') form of the enzyme has been found to decay as a triple exponential with time consts. and relative contributions of 5.4 ns (74.3%), 2.2 ns (20.4%) and 0.4 ns (5.3%). A comparison of recovered decay parameters obtained for native and mutated *H. lanuginosa* lipase shows that Trp89 contributes about 61% to the class of emitting species with the lifetime of 5.4 ns. The fluorescence quenching data show that three out of four tryptophans (i.e., 117, 221 and 260 residues) within *H. lanuginosa* lipase are totally quenchable by acrylamide while completely inaccessible to iodide. On the contrary, the Trp89 residue is available for both quenchers. Using steady-state iodide fluorescence quenching data and the fluorescence-quenching-resolved-spectra (FQRS) method, the total emission spectrum of the native lipase has been decomposed into two spectral components. One of them, unquenchable by iodide, has a maximum of fluorescence emission at 330 nm and the second one, exposed to the solvent, emits at 338 nm. The resolved spectrum of the redder component corresponds to the Trp89 residue, which participates in about 65% of the total *H. lanuginosa* emission. The dynamic Stern-Volmer quenching consts. calculated for both native ('closed-lid') and inhibited ('open-lid') lipase are 2.71 and 4.49 M<sup>-1</sup>, resp. The values obtained indicate that Trp89 is not deeply buried in the protein matrix. Our results suggest that distinct configurations of fungal lipase can be monitored using the fluorescence of the Trp89 residue located in the 'lid'-helix which participates in an interfacial activation of the enzyme.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L50 ANSWER 11 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1998:587613 SCISEARCH Full-text

THE GENUINE ARTICLE: 106NT

TITLE: Anatomy of lipase binding sites: the scissile  
fatty acid binding site

AUTHOR: Pleiss J; Fischer M; Schmid R D (Reprint)

CORPORATE SOURCE: Univ Stuttgart, Inst Tech Biochem, Allmandring 31, D-70569 Stuttgart, Germany (Reprint); Univ Stuttgart, Inst Tech Biochem, D-70569 Stuttgart, Germany  
COUNTRY OF AUTHOR: Germany  
SOURCE: CHEMISTRY AND PHYSICS OF LIPIDS, (JUN 1998) Vol. 93, No. 1-2, pp. 67-80.  
ISSN: 0009-3084.  
PUBLISHER: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 41  
ENTRY DATE: Entered STN: 1998  
Last Updated on STN: 1998

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Shape and physico-chemical properties of the scissile fatty acid binding sites of six lipases and two serine esterases were analyzed and compared in order to understand the molecular basis of substrate specificity. All eight serine esterases and lipases have similar architecture and catalytic mechanism of ester hydrolysis, but different substrate specificities for the acyl moiety. Lipases and esterases differ in the geometry of their binding sites, lipases have a large, hydrophobic scissile fatty acid binding site, esterases like acetylcholinesterase and bromoperoxidase have a small acyl binding pocket, which fits exactly to their favorite substrates. The lipases were subdivided into three sub-groups: (1) lipases with a hydrophobic, crevice-like binding site located near the protein surface (lipases from *Rhizomucor* and *Rhizopus*); (2) lipases with a funnel-like binding site (lipases from *Candida antarctica*, *Pseudomonas* and mammalian pancreas and cutinase); and (3) lipases with a tunnel-like binding site (lipase from *Candida rugosa*). The length of the scissile fatty acid binding site varies considerably among the lipases between 7.8 Angstrom in cutinase and 22 Angstrom in *Candida rugosa* and *Rhizomucor miehei* lipase. Location and properties of the scissile fatty acid binding sites of all lipases of known structure were characterized. Our model also identifies the residues which mediate chain length specificity and thus may guide protein engineering of lipases for changed chain length specificity. The model was supported by published experimental data on the chain length specificity profile of various lipases and on mutants of fungal lipases with changed fatty acid chain length specificity. (C) 1998 Elsevier Science Ireland Ltd. All rights reserved.

L50 ANSWER 12 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:587612 SCISEARCH Full-text  
THE GENUINE ARTICLE: 106NT  
TITLE: Insights into the molecular basis for fatty acyl specificities of lipases from *Geotrichum candidum* and *Candida rugosa*  
AUTHOR: Holmquist M (Reprint)  
CORPORATE SOURCE: Royal Inst Technol, Dept Biochem & Biotechnol, SE-10044 Stockholm, Sweden (Reprint)  
COUNTRY OF AUTHOR: Sweden  
SOURCE: CHEMISTRY AND PHYSICS OF LIPIDS, (JUN 1998) Vol. 93, No. 1-2, pp. 57-66.  
ISSN: 0009-3084.  
PUBLISHER: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 49  
ENTRY DATE: Entered STN: 1998  
Last Updated on STN: 1998

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Despite immense progress in our comprehension of lipase structure and function during the past decade, the basis for lipase acyl specificities has remained poorly understood. This review summarizes some recent advances in the understanding at the molecular-level of substrate acyl recognition by two members in a group of large (M-w similar to 60 kDa) microbial lipases. Two aspects of acyl specificity will be focused upon. (1) The unique preference of a fungal *Geotrichum candidum* lipase for long-chain cis ( $\Delta^9$ ) unsaturated fatty acid moieties in the substrate. Mutational analysis of this lipase identified residues essential for its anomalous acyl preference. This information highlighted for the first time parts in the lipase molecule involved in substrate acyl differentiation. These results are discussed in the context of the 3D-structure of a *G. candidum* lipase isoenzyme and structures of the related *Candida rugosa* lipase in complex with inhibitors. (ii) The mechanism by which the yeast *C. rugosa* lipase discriminates between enantiomers of a substrate with a chiral acyl moiety. Molecular modeling in combination with substrate engineering and kinetic analyses, identified two alternative substrate binding modes. This allowed for the proposal of a molecular mechanism explaining how long-chain alcohols can

act as enantioselective inhibitors of this enzyme. A picture is thus beginning to emerge of the interplay between lipase structure and fatty acyl specificity. (C) 1998 Elsevier Science Ireland Ltd. All rights reserved.

L50 ANSWER 13 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 5

ACCESSION NUMBER: 1998:455633 CAPLUS Full-text  
DOCUMENT NUMBER: 129:213493  
TITLE: The decay of the fluorescence anisotropy of tryptophan residues in fungal lipase from *Humicola lanuginosa*  
AUTHOR(S): Stobiecka, A.; Wysocki, S.  
CORPORATE SOURCE: Institute General Food Chemistry, Technical University Lodz, Lodz, 90924, Pol.  
SOURCE: Journal of Radioanalytical and Nuclear Chemistry (1998), 232(1-2), 43-48  
CODEN: JRNCDM; ISSN: 0236-5731  
PUBLISHER: Elsevier Science S.A.  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Pulse nanosecond fluorescence anisotropy decay was used to study the mobility of Trp residues within fungal lipase from *Humicola lanuginosa*. The decay of emission anisotropy of protein in native, inhibited, and mutated form was investigated in buffered water and 50% volume/volume glycerol solns. The rotational motions of the lipase were analyzed in terms of 2 different kinetic models. The fluorescence emission anisotropy decay can best be described with 2 rotational correlation times : 0.63 and 5.45 ns in water and 0.98 and 10.70 ns and in 50% volume/volume glycerol solution. The decay of inhibited and mutated *H. lanuginosa* lipase showed a similar biexponential character. These results are interpreted in terms of local or segmental motion arising from a mass of about 1083 Da which corresponds to the 'lid'-helix fragment of the enzyme.

L50 ANSWER 14 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:587609 SCISEARCH Full-text  
THE GENUINE ARTICLE: 106NT  
TITLE: Biochemical properties of staphylococcal (phospho) lipases  
AUTHOR: Simons J W F A; Gotz F; Egmond M R; Verheij H M (Reprint)  
CORPORATE SOURCE: Univ Utrecht, Inst Biomembranes, Ctr biomembranes & Lipid Enzymol, Dept Enzymol & Prot Engr, POB 80054, Padualaan 8, NL-3508 TB Utrecht, Netherlands (Reprint); Univ Utrecht, Inst Biomembranes, Ctr biomembranes & Lipid Enzymol, Dept Enzymol & Prot Engr, NL-3508 TB Utrecht, Netherlands; Univ Tubingen, Tubingen, Germany  
COUNTRY OF AUTHOR: Netherlands; Germany  
SOURCE: CHEMISTRY AND PHYSICS OF LIPIDS, (JUN 1998) Vol. 93, No. 1-2, pp. 27-37.  
ISSN: 0009-3084.  
PUBLISHER: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 37  
ENTRY DATE: Entered STN: 1998  
Last Updated on STN: 1998

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Various staphylococci secrete lipases which require calcium ions for activity, and have profound preferences for substrates with different chain lengths. The lipase from *Staphylococcus hyicus* is exceptional since it has higher phospholipase than lipase activity. This paper gives an overview of the biochemical properties of these enzymes. It appears that chain length selectivity of these enzymes resides in the acylation step. Interfaces mainly influence the acylation step. Calcium ions do not influence the rate of acylation or deacylation although stabilise the enzyme against denaturation. Molecular modelling based on the X-ray structure of *Pseudomonas glumae* lipase was used to construct a model of the staphylococcal lipases. With this model the position of several residues involved in substrate selectivity was predicted. Moreover, a sequence element could be assigned that may function as the so-called lid domain in staphylococcal lipases. Sequence alignment of four staphylococcal lipases, and lipases from *P. glumae* and *Bacillus thermocatenulatus* identified several potential calcium ligands, one of which was verified by site directed mutagenesis. It is concluded that stabilisation of lipases by calcium ions might be a more general phenomenon than recognized so far. (C) 1998 Elsevier Science Ireland Ltd. All rights reserved.

L50 ANSWER 15 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1997:757085 CAPLUS Full-text  
DOCUMENT NUMBER: 128:24317  
TITLE: Detergent compositions containing a specific  
lipolytic enzyme and alkyl  
polyglycoside surfactant  
INVENTOR(S): Baeck, Andre Cesar; Kasturi, Chandrika  
PATENT ASSIGNEE(S): Procter and Gamble Company, USA; Baeck, Andre Cesar;  
Kasturi, Chandrika  
SOURCE: PCT Int. Appl., 74 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9743377	A1	19971120	WO 1996-US7088	19960515
W:	AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
AU 9658618	A	19971205	AU 1996-58618	19960515
EP 912683	A1	19990506	EP 1996-920251	19960515
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI			
BR 9612608	A	19990720	BR 1996-12608	19960515
PRIORITY APPLN. INFO.:			WO 1996-US7088	W 19960515

AB The present invention relates to detergent compns. containing a variants of the native lipolytic enzyme producible by Humicola lanuginosa and Thermomyces lanuginosus or by cloning and expressing the gene responsible for producing the variants into a host organism and an ROGx surfactant (R = C10-16 alkyl, G = reducing saccharide containing 5-6 C atoms, x = 1-3). Such compns. provide improved overall detergency performance: reduced redeposition of greasy/oily substances on fabrics, dishware and hard surfaces thereby improving whiteness maintenance, cleaning, spotting, filming and stain removal performances.

L50 ANSWER 16 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1997:757082 CAPLUS Full-text  
DOCUMENT NUMBER: 128:36417  
TITLE: Detergent compositions comprising specific  
lipolytic enzyme and a soil release  
polymer  
INVENTOR(S): Baeck, Andre Cesar; Kasturi, Chandrika  
PATENT ASSIGNEE(S): Procter and Gamble Company, USA; Baeck, Andre Cesar;  
Kasturi, Chandrika  
SOURCE: PCT Int. Appl., 77 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9743374	A1	19971120	WO 1996-US7085	19960515
W:	AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
CA 2254919	A1	19971120	CA 1996-2254919	19960515
AU 9657957	A	19971205	AU 1996-57957	19960515
BR 9612614	A	19990720	BR 1996-12614	19960515
EP 931134	A1	19990728	EP 1996-914666	19960515
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI			
PRIORITY APPLN. INFO.:			WO 1996-US7085	A 19960515

AB Lipolytic enzymes are variants of the native lipolytic enzymes producible by Humicola lanuginosa and Thermomyces lanuginosus. A soil release polymer is selected from sulfonated poly(ethoxy)/(propoxy)-end capped ester oligomers and/or short block polymers manufactured from di-Me terephthalate (I), 1,2-propylene glycol (II), Me-capped polyethylene glycol and/or short block polymers manufactured from I, II, and sulfoethoxylates. Thus, a granular detergent contained a Na linear C12-alkyl benzenesulfonate 22, a phosphate 23, a carbonate 23, a silicate 14, Zeolite A 8.2, Dequest 2060 0.4, Na sulfate 5.5, amylase 0.005, protease 0.01, pectinase 0.02, Lipolase Ultra 0.005, a soil release polymer 0.2, cellulase 0.001, and water and minors to 100%.

L50 ANSWER 17 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1997:220628 CAPLUS Full-text

DOCUMENT NUMBER: 126:208956

TITLE: Recombinant lipases with C- and/or N-terminal extensions and their use in detergents

INVENTOR(S): Fuglsang, Claus Crone; Okkels, Jens Sigurd; Pertersen, Dorte Aaby; Patkar, Shamkant Anant; Thellersen, Marianne; Vind, Jesper; Halkier, Torben; Joergensen, Steen Troels; et al.

PATENT ASSIGNEE(S): Novo Nordisk A/s, Den.; Fuglsang, Claus Crone; Okkels, Jens Sigurd; Pertersen, Dorte Aaby; Patkar, Shamkant Anant; Thellersen, Marianne; Vind, Jesper; Halkier, Torben

SOURCE: PCT Int. Appl., 197 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 4

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9704078	A1	19970206	WO 1996-DK321	19960712
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA				
AU 9664140	A	19970218	AU 1996-64140	19960712
CN 1193346	A	19980916	CN 1996-196371	19960712
PRIORITY APPLN. INFO.:			DK 1995-832	A 19950714
			DK 1995-1013	A 19950913
			DK 1995-1096	A 19950929
			DK 1995-1306	A 19951121
			US 1996-11634P	P 19960214
			DK 1996-372	A 19960401
			US 1996-20461P	P 19960507
			WO 1996-DK321	W 19960712

AB The invention relates to a modified enzyme with lipolytic activity recovered from a filamentous fungi or a bacteria having one or more peptide addns. at the N-terminal and/or the C-terminal ends in comparison to the parent enzyme. The peptide addns. significantly improve the washing performance of the lipase. Further, the invention relates to a DNA sequence encoding said modified enzyme, a vector comprising said DNA sequence, a host cell harboring said DNA sequence or said vector, and a process for producing said modified enzyme with lipolytic activity. The lipase variants are useful in detergent compns. Numerous lipase variants containing substitution mutations and C- and/or N-terminal addns. were prepared with recombinant Aspergillus oryzae or with Escherichia coli. The addition of SPIRR to the N-terminus of Humicola lanuginosa lipase increased the wash performance relative to the parent enzyme twofold.

L50 ANSWER 18 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1997:897558 SCISEARCH Full-text

THE GENUINE ARTICLE: YJ709

TITLE: Identification of residues essential for differential fatty acyl specificity of Geotrichum candidum lipases I and II

AUTHOR: Holmquist M (Reprint); Tessier D C; Cygler M

CORPORATE SOURCE: NATL RES COUNCIL CANADA, BIOTECHNOL RES INST, MONTREAL, PQ H4P 2R2, CANADA; ROYAL INST TECHNOL, DEPT BIOCHEM & BIOTECHNOL, S-10044 STOCKHOLM, SWEDEN

COUNTRY OF AUTHOR: CANADA; SWEDEN

SOURCE: BIOCHEMISTRY, (2 DEC 1997) Vol. 36, No. 48, pp. 15019-15025.

ISSN: 0006-2960.  
PUBLISHER: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 44  
ENTRY DATE: Entered STN: 1997  
Last Updated on STN: 1997

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The fungus *Geotrichum candidum* produces two lipase isoenzymes, GCL I and GCL II, with distinct differences in substrate specificity despite their 86% identical primary structure. GCL I prefers ester substrates with long-chain cis ( $\Delta^9$ ) unsaturated fatty acid moieties, whereas GCL II also accepts medium-length (C8-C14) acyl moieties in the substrate. To reveal structural elements responsible for differences in substrate differentiating ability of these isoenzymes, we designed, expressed, and characterized 12 recombinant lipase variants. Three chimeric lipases containing unique portions of the N-terminal and the C-terminal part of GCL I and GCL II, respectively, were constructed and enzymatically characterized. Activities were measured against mixed triglyceride-poly(dimethyl siloxane) particles. Our results indicate that residues within sequence positions 349-406 are essential for GCL I's high triolein/trioctanoin activity ratio of 20. The substitution of that segment in the specific GCL I to the corresponding residues in the nonspecific GCL II, resulted in an enzyme with a triolein/trioctanoin activity ratio of 1.4, identical to that of GCL II. The reverse mutation in GCL II increased its specificity for triolein by a factor of 2, thus only in part restoring the high specificity seen with GCL I. In further experiments, the point mutations at the active site entrance of the GCL I, Leu358Phe and Ile357Ala/Leu358Phe, lowered the triolein/trioctanoin activity ratio from 20 to 4 and 2.5, respectively. The substitutions Cys379Phe/Ser380Tyr at the bottom of the active site cavity of GCL I decreased its specificity to a value of 3.6. Measurements of lipase activity with substrate particles composed of pure triglycerides or ethyl esters of oleic and octanoic acids resulted in qualitatively similar results as reported above. Our data reveal for the first time the identity of residues essential for the unusual substrate preference of GCL I and show that the anatomy, both at the entrance and the bottom of the active site cavity, plays a key role in substrate discrimination.

L50 ANSWER 19 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 6

ACCESSION NUMBER: 1997:372120 CAPLUS Full-text  
DOCUMENT NUMBER: 126:327220  
TITLE: Comparison of the adsorption and activity of lipases from *Humicola lanuginosa* and *Candida antarctica* on solid surfaces  
AUTHOR(S): Wannerberger, Kristin; Arnebrant, Thomas  
CORPORATE SOURCE: Department of Food Technology, University of Lund, Lund, S-221 00, Swed.  
SOURCE: Langmuir (1997), 13(13), 3488-3493  
CODEN: LANGD5; ISSN: 0743-7463  
PUBLISHER: American Chemical Society  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The adsorption of lipases from *H. lanuginosa*, the wild type (WT) and a mutant with increased hydrophobicity in the active site region, and lipase B from *C. antarctica* to solid surfaces was studied by in situ ellipsometry. In addition, the activity of the adsorbed lipase was measured in situ and from the different surface concns., the specific activity was calculated. Concentration- and temperature-dependence as well as the influence of surface wettability was studied using silica surfaces with varying degrees of methylation. The higher hydrophobicity of the mutant compared to the WT resulted in increased amts. adsorbed, no desorbable fraction during rinsing, and absence of an initial maximum in adsorbed amount (as seen for the WT) at higher concns. No temperature dependence for the mutant could be observed. This was in contrast to the WT where both the plateau value of the adsorbed amount and the activity decreased with increasing temperature. The influence of surface wettability was similar for both featuring a decreased adsorbed amount and increased specific activity with increasing wettability. The amount adsorbed of the *Candida* lipase was significantly higher at all concns., compared to the other lipases, and the activity was very low, indicating adsorption with the active site region directed toward the surface. The surface wettability did not affect the activity of *Candida* lipase.

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L50 ANSWER 20 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1997:548646 CAPLUS Full-text  
DOCUMENT NUMBER: 127:189671  
TITLE: Biotechnology in detergents and cosmetics  
AUTHOR(S): Ito, Susumu  
CORPORATE SOURCE: Tochigi Res. Lab., Kao Corp., Tochigi, 321-34, Japan  
SOURCE: *Baio* (1997), 55(8), 541-545  
CODEN: BIDSE6; ISSN: 0914-8981



PUBLISHER: Baioindasutori Kyokai  
DOCUMENT TYPE: Journal; General Review  
LANGUAGE: Japanese

AB A review with 18 refs. on site-directed mutation of subtilisin suitable for detergents, heat-stable mutation of *Bacillus licheniformis* amylase, amylopullulanase, site-directed mutation of  $\alpha$ -amylase to improve durability against chelating agents and heat, alkaline cellulase of alkalophilic *Bacillus*, cellulase of *Aspergillus oryzae*, production of lipase of *Humicola lanuginosa* by *A. oryzae* as host organism, catalytic site of *Mocor miehei* enzyme, raw materials for cosmetics, sophorolipid, kojic acid, hyaluronate,  $\gamma$ -linolenate, shikonin, and tuberose polysaccharide.

L50 ANSWER 21 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1997:250761 SCISEARCH Full-text

THE GENUINE ARTICLE: WP714

TITLE: Computational studies of the activation of lipases  
and the effect of a hydrophobic environment

AUTHOR: Peters G H (Reprint); Toxvaerd S; Olsen O H; Svendsen A

CORPORATE SOURCE: UNIV COPENHAGEN, HC ORSTED INST, DEPT CHEM 3, UNIV PK 5,  
DK-2100 COPENHAGEN O, DENMARK (Reprint); NOVO NORDISK AS,  
DK-2880 BAGSVAERD, DENMARK

COUNTRY OF AUTHOR: DENMARK

SOURCE: PROTEIN ENGINEERING, (FEB 1997) Vol. 10, No. 2, pp.  
137-147.

ISSN: 0269-2139.

PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD, ENGLAND OX2  
6DP.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 69

ENTRY DATE: Entered STN: 1997

Last Updated on STN: 1997

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB We have investigated the activation pathway of three wild type lipases and three mutants using molecular dynamics techniques combined with a constrained mechanical protocol. The activation of these lipases involves a rigid body hinge-type motion of a single helix, which is displaced during activation to expose the active site and give access to the substrate. Our results suggest that the activation of lipases is enhanced in a hydrophobic environment as is generally observed in experiments. The energy gain upon activation varies between the different lipases and depends strongly on the distribution of the charged residues in the activating loop region. In a low dielectric constant medium (such as a lipid environment), the electrostatic interactions between the residues located in the vicinity of the activating loop (lipid contact zone) are dominant and determine the activation of the lipases. Calculations of the pK(a)s qualitatively indicate that some titratable residues experience significant pK shifts upon activation. These calculations may provide sufficient details for an understanding of the origin and magnitude of a given electrostatic effect and may provide an avenue for exploring the activation pathway of lipases.

L50 ANSWER 22 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1997:170960 SCISEARCH Full-text

THE GENUINE ARTICLE: WJ876

TITLE: Altered acyl chain length specificity of *Rhizopus delemar*  
lipase through mutagenesis and molecular  
modeling

AUTHOR: Klein R R (Reprint); King G; Moreau R A; Haas M J

CORPORATE SOURCE: USDA ARS, ERRC, 600 E MERMAID LANE, WYNDMOOR, PA 19038  
(Reprint)

COUNTRY OF AUTHOR: USA

SOURCE: LIPIDS, (FEB 1997) Vol. 32, No. 2, pp. 123-130.

ISSN: 0024-4201.

PUBLISHER: AMER OIL CHEMISTS SOC, 1608 BROADMOOR DRIVE, CHAMPAIGN, IL  
61821-0489.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE; AGRI

LANGUAGE: English

REFERENCE COUNT: 27

ENTRY DATE: Entered STN: 1997

Last Updated on STN: 1997

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB

The acyl binding site of *Rhizopus delemar* prolipase and mature lipase was altered through site-directed mutagenesis to improve lipase specificity for short- or medium-chain length fatty acids. Computer-generated structural models of *R. delemar* lipase were used in mutant protein design and in the interpretation of the catalytic properties of the resulting recombinant enzymes. Molecular dynamics simulations of the double mutant, val209trp + phe112trp, predicted that the introduction of trp112 and trp209 in the acyl binding groove would sterically hinder the docking of fatty acids longer than butyric acid. Assayed against a mixture of triacylglycerol substrates, the val209trp + phe112trp mature lipase mutant showed an 80-fold increase in the hydrolysis of tributyrin relative to the hydrolysis of tricaprylin while no triolein hydrolysis was detected. By comparison, the val94Trp mutant, predicted to pose steric or geometric constraints for docking fatty acids longer than caprylic acid in the acyl binding groove, resulted in a modest 1.4-fold increase in tricaprylin hydrolysis relative to the hydrolysis of tributyrin. Molecular models of the double mutant phe95asp + phe214arg indicated the creation of a salt bridge between asp95 and arg214 across the distal end of the acyl binding groove. When challenged with a mixture of triacylglycerols, the phe95asp + phe214arg substitutions resulted in an enzyme with 3-fold enhanced relative activity for tricaprylin compared to triolein, suggesting that structural determinants for medium-chain length specificity may reside in the distal end of the acyl binding groove. Attempts to introduce a salt bridge within 8 Angstrom of the active site by the double mutation leu146lys + ser115asp destroyed catalytic activity entirely. Similarly, the substitution of polar Gln at the rim of the acyl binding groove for phe112 largely eliminated catalytic activity of the lipase.

L50 ANSWER 23 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1997:470292 SCISEARCH Full-text  
 THE GENUINE ARTICLE: XF115  
 TITLE: Computer-aided modelling of stereoselective triglyceride hydrolysis catalyzed by *Rhizopus oryzae* lipase  
 AUTHOR: Holzwarth H C (Reprint); Pleiss J; Schmid R D  
 CORPORATE SOURCE: UNIV STUTTGART, INST TECH BIOCHEM, D-70569 STUTTGART, GERMANY  
 COUNTRY OF AUTHOR: GERMANY  
 SOURCE: JOURNAL OF MOLECULAR CATALYSIS B-ENZYMATIC, (10 JUN 1997) Vol. 3, No. 1-4, pp. 73-82.  
 ISSN: 1381-1177.  
 PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.  
 DOCUMENT TYPE: Article; Journal  
 FILE SEGMENT: PHYS  
 LANGUAGE: English  
 REFERENCE COUNT: 28  
 ENTRY DATE: Entered STN: 1997  
 Last Updated on STN: 1997

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB

Lipase from *Rhizopus oryzae* catalyzes the stereoselective hydrolysis of triglycerides and analogues. Stereopreference and degree of enantiomeric excess of the product varies with the structure of the substrate: trioctanoylglycerol ('ester') and the sn-2 analogues 2-X-1,3-dioctanoylpropandiol, where 2-X = 2-O-octyl ('ether') and 2-hexyl ('alkane'), are preferentially hydrolyzed at sn-1, substitution by 2-phenyl ('phenyl') reverses the stereopreference to sn-3. We have modelled the stereoselectivity of *Rhizopus oryzae* lipase by docking the tetrahedral intermediates of these substrates in two orientations, appropriate to hydrolysis at sn-1 or sn-3, respectively. The initial complexes were further relaxed by molecular dynamics simulations. The favoured orientation of a substrate is characterized by three factors: (1) The substrate fits well into the binding site. The glycerol backbone is relaxed and the sn-2 chain points to a well defined hydrophobic binding site. (2) The oxyanion is stabilized by an extra hydrogen bond from the side chain of Thr 83. (3) The substrate lacks repulsive interactions with protein side chains, especially of Leu 258. Our model is consistent with experimental data and explains qualitatively the ranking of four different substrates with regard to stereoselectivity. It can be used to design lipase mutants with modified stereoselectivity.

L50 ANSWER 24 OF 37 MEDLINE on STN DUPLICATE 7

ACCESSION NUMBER: 97042947 MEDLINE Full-text  
 DOCUMENT NUMBER: PubMed ID: 8888145  
 TITLE: The consequences of engineering an extra disulfide bond in the *Penicillium camembertii* mono- and diglyceride specific lipase.  
 AUTHOR: Yamaguchi S; Takeuchi K; Mase T; Oikawa K; McMullen T; Derewenda U; McElhaney R N; Kay C M; Derewenda Z S  
 CORPORATE SOURCE: Tsukuba Research Laboratories, Amano Pharmaceutical Co. Ltd., Japan.

SOURCE: Protein engineering, (1996 Sep) Vol. 9, No. 9, pp. 789-95.  
Journal code: 8801484. ISSN: 0269-2139.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199705

ENTRY DATE: Entered STN: 14 May 1997  
Last Updated on STN: 14 May 1997  
Entered Medline: 6 May 1997

AB The extracellular lipase from *Penicillium camembertii* has unique substrate specificity restricted to mono- and diglycerides. The enzyme is a member of a homologous family of lipases from filamentous fungi. Four of these proteins, from the fungi *Rhizomucor miehei*, *Humicola lanuginosa*, *Rhizopus delemar* and *P. camembertii*, have had their structures elucidated by X-ray crystallography. In spite of pronounced sequence similarities the enzymes exhibit significant differences. For example, the thermostability of the *P. camembertii* lipase is considerably lower than that of the *H. lanuginosa* enzyme. Since only the *P. camembertii* enzyme lacks the characteristic long disulfide bridge, corresponding to Cys22-Cys268 in the *H. lanuginosa* lipase, we have engineered this disulfide into the former enzyme in the hope of obtaining a significantly more stable fold. The properties of the double mutant (Y22C and G269C) were assessed by a variety of biophysical techniques. The extra disulfide link was found to increase the melting temperature of the protein from 51 to 63 degrees C. However, no difference is observed under reducing conditions, indicating an intrinsic instability of the new disulfide. The optimal temperature for catalytic activity decreased by 10 degrees C and the optimum pH was shifted by 0.7 units to more acidic.

L50 ANSWER 25 OF 37 MEDLINE on STN DUPLICATE 8

ACCESSION NUMBER: 97015915 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 8862552

TITLE: The role of Glu87 and Trp89 in the lid of *Humicola lanuginosa* lipase.

AUTHOR: Martinelle M; Holmquist M; Clausen I G; Patkar S; Svendsen A; Hult K

CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.

SOURCE: Protein engineering, (1996 Jun) Vol. 9, No. 6, pp. 519-24.  
Journal code: 8801484. ISSN: 0269-2139.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199612

ENTRY DATE: Entered STN: 28 Jan 1997  
Last Updated on STN: 29 Jan 1999  
Entered Medline: 9 Dec 1996

AB The importance of Glu87 and Trp89 in the lid of *Humicola lanuginosa* lipase for the hydrolytic activity at the water/lipid interface was investigated by site-directed mutagenesis. It was found that the effect on the hydrolytic activity upon the replacement of Trp89 with Phe, Leu, Gly or Glu was substrate dependent. The Trp89 mutants displayed an altered chain length specificity towards triglycerides, with a higher relative activity towards triacetin and trioctanoin compared with tributyrin. Trp89 was shown to be less important in the hydrolysis of vinyl esters compared with ethyl esters and triglycerides. An exclusive effect on the acylation reaction rate by the mutation of Trp89 was consistent with the data. It is suggested that Trp89 is important in the process of binding the acyl chain of the substrate into the active site for optimal acylation reaction rate. The Trp89Phe mutation resulted in an increased hydrolytic activity towards 2-alkylalkanoic acid esters. This is suggested to be due to reduction of unfavourable van der Waals contacts between Trp89 and the 2-substituent of the substrate. Thus, in contrast to natural substrates, Trp89 has a negative impact on the catalytic efficiency when substrates with bulky acyl chains are used. In contrast to the Trp89 mutations, the effect on the hydrolytic activity of the Glu87Ala mutation was almost substrate independent, 35-70% activity of wild-type lipase. A reduction of both the acylation and deacylation reaction was consistent with the data.

L50 ANSWER 26 OF 37 LIFESCI COPYRIGHT 2007 CSA on STN

ACCESSION NUMBER: 1998:29753 LIFESCI Full-text

TITLE: Acyl glycerol hydrolases: Inhibitors, interface and catalysis

AUTHOR: Cambillau, C.; Longhi, S.; Nicolas, A.; Martinez, C.

CORPORATE SOURCE: Architecture et Fonction des Macromolécules Biologiques, UPR 9039, CNRS, IFRI, 31 Chemin Joseph Aiguier, 13402 Marseille, Cedex 20, France

SOURCE: CURR. OPIN. STRUCT. BIOL., (19960800) vol. 6, no. 4, pp.

449-455.  
ISSN: 0959-440X.  
DOCUMENT TYPE: Journal  
TREATMENT CODE: General Review  
FILE SEGMENT: K  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB The last five years have witnessed the solution of a large number of lipase structures, which has led, among other insights, to the structural interpretation of the interfacial activation phenomenon in terms of 'lid' opening. This interpretation has been extended this year to include phospholipase A2. Recent structural studies on lipases have provided data on the detailed mechanisms underlying the behaviour of lipases: how they bind to inhibitors or substrates, and what interactions occur between their hydrophobic face and hydrophobic molecules, for example. In addition, studies on cutinase point mutants have shed some light on the role of the oxyanion hole in lipolytic catalysis.

L50 ANSWER 27 OF 37 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1996:113975 BIOSIS Full-text  
DOCUMENT NUMBER: PREV199698686110  
TITLE: Contribution of cutinase serine 42 side chain to the stabilization of the oxyanion transition state.  
AUTHOR(S): Nicolas, Anne; Egmond, Maarten; Verrips, C. Theo; De Vlieg, Jakob; Longhi, Sonia; Cambillau, Christian [Reprint author]; Martinez, Chrislaine  
CORPORATE SOURCE: Lab. Cristallographique Cristallisation des Macromolecules Biol., URA1296-CNRS, IFR1, 31 Chemin J. Aiguier, 13402 Marseille cedex 09, France  
SOURCE: Biochemistry, (1996) Vol. 35, No. 2, pp. 398-410.  
CODEN: BICHAW. ISSN: 0006-2960.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 12 Mar 1996  
Last Updated on STN: 10 Jun 1997

AB Cutinase from the fungus *Fusarium solani* pisi is a lipolytic enzyme able to hydrolyze both aggregated and soluble substrates. It therefore provides a powerful tool for probing the mechanisms underlying lipid hydrolysis. Lipolytic enzymes have a catalytic machinery similar to those present in serine proteinases. It is characterized by the triad Ser, His, and Asp (Glu) residues, by an oxyanion binding site that stabilizes the transition state via hydrogen bonds with two main chain amide groups, and possibly by other determinants. It has been suggested on the basis of a covalently bound inhibitor that the cutinase oxyanion hole may consist not only of two main chain amide groups but also of the Ser42 O-gamma side chain. Among the esterases and the serine and the cysteine proteases, only *Streptomyces scabies* esterase, subtilisin, and papain, respectively, have a side chain residue which is involved in the oxyanion hole formation. The position of the cutinase Ser42 side chain is structurally conserved in *Rhizomucor miehei* lipase with Ser82 O-gamma, in *Rhizopus delemar* lipase with Thr83 O-gamma-1, and in *Candida antarctica* B lipase with Thr40 O-gamma-1. To evaluate the increase in the tetrahedral intermediate stability provided by Ser42 O-gamma, we mutated Ser42 into Ala. Furthermore, since the proper orientation of Ser42 O-gamma is directed by Asn84, we mutated Asn84 into Ala, Leu, Asp, and Trp, respectively, to investigate the contribution of this indirect interaction to the stabilization of the oxyanion hole. The S42A mutation resulted in a drastic decrease in the activity (450-fold) without significantly perturbing the three-dimensional structure. The N84A and N84L mutations had milder kinetic effects and did not disrupt the structure of the active site, whereas the N84W and N84D mutations abolished the enzymatic activity due to drastic steric and electrostatic effects, respectively.

L50 ANSWER 28 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1996:404098 CAPLUS Full-text  
DOCUMENT NUMBER: 125:133994  
TITLE: A URA3-promoter deletion in a pYES vector increases the expression level of a fungal lipase in *Saccharomyces cerevisiae*  
AUTHOR(S): Okkels, Jens Sigurd  
CORPORATE SOURCE: Novo Nordisk, Copenhagen, DK-2100, Den.  
SOURCE: Annals of the New York Academy of Sciences (1996), 782(Recombinant DNA Biotechnology III), 202-207  
CODEN: ANYAA9; ISSN: 0077-8923  
PUBLISHER: New York Academy of Sciences  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Expression of a lipase gene from *Humicola lanuginosa* in *Saccharomyces cerevisiae* is increased, allowing large-scale purification. A simple deletion of the URA3 promoter from a *S. cerevisiae* expression plasmid was performed. The promoter-deleted plasmid has an increased expression level

of fungal lipase gene. The deletion probably causes a poor expression of the URA3 selection marker, probably resulting in a higher copy number per cell of the plasmid. This higher copy number can increase the transcript level per cell and thereby the expression level. In the case of the fungal lipase gene, the expression level with defined inoculum is increased at least three-fold. A part of the 2- $\mu$ m origin of the pYES type plasmid was also deleted by the URA3 promoter deletion without affecting transformation frequency. The URA3 promoter can easily be deleted from most pYES type plasmids, since most of these plasmids only contain the two PstI sites that remove the whole URA3 promoter.

L50 ANSWER 29 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 9

ACCESSION NUMBER: 1996:490978 CAPLUS Full-text  
DOCUMENT NUMBER: 125:215670  
TITLE: Lipases from *Humicola lanuginosa*  
adsorbed to hydrophobic surfaces - desorption and  
activity after addition of surfactants  
AUTHOR(S): Wannerberger, Kristin; Arnebrant, Thomas  
CORPORATE SOURCE: Department of Food Technology, University of Lund, Box  
124, Lund, S-22100, Swed.  
SOURCE: Colloids and Surfaces, B: Biointerfaces (1996),  
7(3/4), 153-164  
CODEN: CSBBEQ; ISSN: 0927-7765  
PUBLISHER: Elsevier  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The adsorption of lipase to methylated silica and its subsequent removal upon sequential addition of surfactants were measured in situ by ellipsometry. In addition, the specific activity of adsorbed lipase without, and after, exposure to surfactants was determined. Two lipases from *Humicola lanuginosa* were studied, the wild-type (WT) and a mutant which was modified to increase the hydrophobicity of the active-site region. The surfactants used were SDS, C12E5 and sodium dodecanoate and they were all studied in the concentration range  $\leq$  their critical micelle concentration (cmc). In all expts. a non-desorbable fraction remained at the surface. This fraction was larger, however, for the WT compared to the mutant. The concentration, expressed as a fraction of the cmc, of surfactant inducing the most pronounced desorption was found to vary among the surfactants. Desorption occurred at increasing fractions of the cmc in the order C12E5, sodium dodecanoate and SDS. This was found for both the WT and the mutant. No significant effect of C12E5 or SDS on the specific activities could be observed while the exposure to sodium dodecanoate markedly increased the specific activity of both lipases.

L50 ANSWER 30 OF 37 MEDLINE on STN DUPLICATE 10

ACCESSION NUMBER: 95242058 MEDLINE Full-text  
DOCUMENT NUMBER: PubMed ID: 7536956  
TITLE: The importance of non-charged amino acids in antibody  
binding to *Humicola lanuginosa* lipase.  
AUTHOR: Naver H; Lovborg U  
CORPORATE SOURCE: Novo Nordisk Industrial Biotechnology, Copenhagen, Denmark.  
SOURCE: Scandinavian journal of immunology, (1995 May) Vol. 41, No.  
5, pp. 443-8.  
Journal code: 0323767. ISSN: 0300-9475.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199505  
ENTRY DATE: Entered STN: 5 Jun 1995  
Last Updated on STN: 29 Jan 1999  
Entered Medline: 25 May 1995

AB The antigenicity of 36 *Humicola lanuginosa* lipase (HL) variants, generated by site directed mutagenesis, was compared with that of the unchanged enzyme. Polyclonal antibodies raised against variant lipases were investigated and compared with the antibodies raised against the wild type lipase in an ELISA competition assay. The results showed that exchange of charged amino acids with polar residues in surface epitopes of HL, results in a tighter binding of the antibody to the epitope. Four amino acids (Trp at position 89, Asp at positions 96 and 254 and Phe at position 211) were found to be essential for antibody binding in each their epitope of the wild type enzyme.

L50 ANSWER 31 OF 37 MEDLINE on STN DUPLICATE 11

ACCESSION NUMBER: 95391076 MEDLINE Full-text  
DOCUMENT NUMBER: PubMed ID: 7662109  
TITLE: Probing a functional role of Glu87 and Trp89 in the lid of  
*Humicola lanuginosa* lipase through  
transesterification reactions in organic solvent.  
AUTHOR: Holmquist M; Clausen I G; Patkar S; Svendsen A; Hult K

CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal  
Institute of Technology, Stockholm, Sweden.  
SOURCE: Journal of protein chemistry, (1995 May) Vol. 14, No. 4,  
pp. 217-24.  
Journal code: 8217321. ISSN: 0277-8033.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199510  
ENTRY DATE: Entered STN: 20 Oct 1995  
Last Updated on STN: 29 Jan 1999  
Entered Medline: 10 Oct 1995

AB To reveal the functional role of Glu87 and Trp89 in the lid of *Humicola lanuginosa* lipase, site-directed mutagenesis at Glu87 and Trp89 was carried out. The catalytic performance of wild-type and mutated lipases was studied in transesterification reactions in cyclohexane at a controlled water activity. Two different acyl donors were used in the investigation: tributyrin, a natural substrate for a lipase, and vinyl butyrate, an activated ester suitable for fast and efficient lipase-catalyzed transformations in preparative organic synthesis. As acyl acceptor 1-heptanol was used. The Glu87Ala mutation decreased the  $V_{max,app}$  value with tributyrin and vinyl butyrate by a factor of 1.5 and 2, respectively. The  $K_{m,app}$  for tributyrin was not affected by the Glu87Ala mutation, but the  $K_{m,app}$  for vinyl butyrate increased twofold compared to the wild-type lipase. Changing Trp89 into a Phe residue afforded an enzyme with a 2.7- and 2-fold decreased  $V_{max,app}$  with the substrates tributyrin and vinyl butyrate, respectively, compared to the wild-type lipase. No significant effects on the  $K_{m,app}$  values for tributyrin or vinyl butyrate were seen as a result of the Trp89Phe mutation. However, the introduction of a Glu residue at position 89 in the lid increased the  $K_{m,app}$  for tributyrin and vinyl butyrate by a factor of > 5 and 2, respectively. The Trp89Glu mutated lipase could not be saturated with tributyrin within the experimental conditions (0-680 mM) studied here. With vinyl butyrate as a substrate the  $V_{max,app}$  was only 6% of that obtained with wild-type enzyme.

L50 ANSWER 32 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1995:340822 CAPLUS Full-text  
DOCUMENT NUMBER: 122:155213  
TITLE: Lipase variants, their manufacture  
with recombinant cells, and their use in detergent  
compositions  
INVENTOR(S): Svendsen, Allan; Patkar, Shamkant Anant; Gormsen,  
Erik; Clausen, Ib Groth  
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.  
SOURCE: PCT Int. Appl., 44 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 5  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9425577	A1	19941110	WO 1994-DK162	19940422
W: AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KG, KP, KR, KZ, LK, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, TJ, UA, US, UZ, VN				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9465358	A	19941121	AU 1994-65358	19940422
BR 9406384	A	19960116	BR 1994-6384	19940422
EP 695348	A1	19960207	EP 1994-913051	19940422
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
CN 1124039	A	19960605	CN 1994-192175	19940422
JP 08509364	T	19961008	JP 1994-523626	19940422
FI 9505018	A	19951020	FI 1995-5018	19951020
PRIORITY APPLN. INFO.:			DK 1993-466	A 19930423
			WO 1994-DK162	W 19940422

AB Novel lipase variants with improved properties, DNA constructs coding for the expression of these variants, host cells expressing the lipase variant genes, and methods for preparing the variants by culturing the recombinant cells are claimed. Lipases comprising a trypsin-like catalytic triad including an active serine located in a predominantly hydrophobic, elongated binding pocket of the lipase mol. are mutated so as to substitute a non-aromatic amino acid residue of a lipid contact zone comprising residues located within the part of the lipase structure containing the active serine residue, which residues may participate in the interaction with the substrate at or during hydrolysis, with an aromatic amino acid residue. Several *Humicola lanuginosa* lipase variants were produced with *Aspergillus oryzae* and tested for wash performance. The [Trp-96]-, and [Trp-96,Asn-210]- lipase variants exhibited 2.7 and 3.4-fold better wash performance than wild-type lipase.

L50 ANSWER 33 OF 37 MEDLINE on STN DUPLICATE 12

ACCESSION NUMBER: 95115502 MEDLINE Full-text  
DOCUMENT NUMBER: PubMed ID: 7815893  
TITLE: Trp89 in the lid of Humicola lanuginosa  
lipase is important for efficient hydrolysis of  
tributylin.  
AUTHOR: Holmquist M; Martinelle M; Clausen I G; Patkar S; Svendsen  
A; Hult K  
CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal  
Institute of Technology, Stockholm, Sweden.  
SOURCE: Lipids, (1994 Sep) Vol. 29, No. 9, pp. 599-603.  
Journal code: 0060450. ISSN: 0024-4201.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199502  
ENTRY DATE: Entered STN: 17 Feb 1995  
Last Updated on STN: 29 Jan 1999  
Entered Medline: 9 Feb 1995

AB To determine whether Trp89 located in the lid of the lipase (EC 3.1.1.3) from Humicola lanuginosa is important for the catalytic property of the enzyme, site-directed mutagenesis at Trp89 was carried out. The kinetic properties of wild type and mutated enzymes were studied with tributyrin as substrate. Lipase variants in which Trp89 was changed to Phe, Leu, Gly or Glu all showed less than 14% of the activity compared to that of the wild type lipase. The Trp89Glu mutant was the least active with only 1% of the activity seen with the wild type enzyme. All Trp mutants had the same binding affinity to the tributyrin substrate interface as did the wild type enzyme. Wild type lipase showed saturation kinetics against tributyrin when activities were measured with mixed emulsions containing different proportions of tributyrin and the nonionic alkyl polyoxyethylene ether surfactant, Triton DF-16. Wild type enzyme showed a  $V_{max} = 6000 \pm 300$  mmol.min<sup>-1</sup>.g<sup>-1</sup> and an apparent  $K_m = 16 \pm 2\%$  (vol/vol) for tributyrin in Triton DF-16, while the mutants did not show saturation kinetics in an identical assay. The apparent  $K_m$  for tributyrin in Triton DF-16 was increased as the result of replacing Trp89 with other residues (Phe, Leu, Gly or Glu). The activities of all mutants were more sensitive to the presence of Triton DF-16 in the tributyrin substrate than was wild type lipase. The activity of the Trp89Glu mutant was decreased to 50% in the presence of 2 vol% Triton DF-16 compared to the activity seen with pure tributyrin as substrate. (ABSTRACT TRUNCATED AT 250 WORDS)

L50 ANSWER 34 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1995:18786 CAPLUS Full-text  
DOCUMENT NUMBER: 122:34002  
TITLE: Detergent compositions comprising lipase  
variants  
AUTHOR(S): Novo, Nordisk  
CORPORATE SOURCE: Den.  
SOURCE: Research Disclosure (1994), 359, 151-6 (No. 35944)  
CODEN: RSDSBB; ISSN: 0374-4353  
DOCUMENT TYPE: Journal; Patent  
LANGUAGE: English  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
-----	---	-----	-----	-----
RD 359044		19940310		

PRIORITY APPLN. INFO.: RD 1994-359044 19940310

AB Detergent compns., especially for dishwashing, contain lipase from Humicola lanuginosa for improved effectiveness.

L50 ANSWER 35 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1993:576669 CAPLUS Full-text  
DOCUMENT NUMBER: 119:176669  
TITLE: Improving the resistance of proteins and enzymes to  
proteolysis by manipulation of surface structures  
INVENTOR(S): Svendsen, Allan; Clausen, Ib Groth; Pathar, Shamkant  
Anant  
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.  
SOURCE: PCT Int. Appl., 55 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9311254	A1	19930610	WO 1992-DK351	19921126
W: BR, CA, FI, JP, KR, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
JP 07504807	T	19950601	JP 1992-509724	19921126
EP 667915	A1	19950823	EP 1992-924587	19921126
R: AT, BE, DE, DK, ES, FR, GB, IT, NL				
BR 9206815	A	19951031	BR 1992-6815	19921126
FI 9402467	A	19940526	FI 1994-2467	19940526
PRIORITY APPLN. INFO.:				
			WO 1991-DK350	A 19911126
			DK 1950-91003	A 19911126
			DK 1950-92003	A 19911126
			WO 1992-DK351	W 19921126

AB Enzymes or other proteins are made more resistant to proteinases by modification of the sequences of surface loops that are common sites of proteolysis. The method is particularly suited for enzymes to be used in proteinase-containing laundry detergents. These sites are identified by very limited proteolysis of the protein and N-terminal sequencing of the cleavage products. Three cleavage sites identified the lipase of *Humicola lanuginosa* DSM 4106 at positions 162-165, 209-212, and 239-242 were substituted with an array of tetrapeptides by standard methods of site-directed mutagenesis of the cloned gene and the proteins manufactured by expression of the gene in *Aspergillus oryzae*. Under conditions in which the wild-type proteinase lost 75% of its activity in 90 min upon digestion with Savinase® (subtilisin), one of these variants retained >80% of its activity.

L50 ANSWER 36 OF 37 MEDLINE on STN DUPLICATE 13  
ACCESSION NUMBER: 94183411 MEDLINE Full-text  
DOCUMENT NUMBER: PubMed ID: 8136025  
TITLE: Lipases from *Rhizomucor miehei* and *Humicola lanuginosa*: modification of the lid covering the active site alters enantioselectivity.  
AUTHOR: Holmquist M; Martinelle M; Berglund P; Clausen I G; Patkar S; Svendsen A; Hult K  
CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.  
SOURCE: Journal of protein chemistry, (1993 Dec) Vol. 12, No. 6, pp. 749-57.  
Journal code: 8217321. ISSN: 0277-8033.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: (COMPARATIVE STUDY)  
Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199404  
ENTRY DATE: Entered STN: 9 May 1994  
Last Updated on STN: 29 Jan 1999  
Entered Medline: 28 Apr 1994

AB The homologous lipases from *Rhizomucor miehei* and *Humicola lanuginosa* showed approximately the same enantioselectivity when 2-methyldecanoic acid esters were used as substrates. Both lipases preferentially hydrolyzed the S-enantiomer of 1-heptyl 2-methyldecanoate (*R. miehei*: ES = 8.5; *H. lanuginosa*: ES = 10.5), but the R-enantiomer of phenyl 2-methyldecanoate (ER = 2.9). Chemical arginine specific modification of the *R. miehei* lipase with 1,2-cyclohexanedione resulted in a decreased enantioselectivity (ER = 2.0), only when the phenyl ester was used as a substrate. In contrast, treatment with phenylglyoxal showed a decreased enantioselectivity (ES = 2.5) only when the heptyl ester was used as a substrate. The presence of guanidine, an arginine side chain analog, decreased the enantioselectivity with the heptyl ester (ES = 1.9) and increased the enantioselectivity with the aromatic ester (ER = 4.4) as substrates. The mutation, Glu 87 Ala, in the lid of the *H. lanuginosa* lipase, which might decrease the electrostatic stabilization of the open-lid conformation of the lipase, resulted in 47% activity compared to the native lipase, in a tributyrin assay. The Glu 87 Ala mutant showed an increased enantioselectivity with the heptyl ester (ES = 17.4) and a decreased enantioselectivity with the phenyl ester (ER = 2.5) as substrates, compared to native lipase. The enantioselectivities of both lipases in the esterification of 2-methyldecanoic acid with 1-heptanol were unaffected by the lid modifications.

L50 ANSWER 37 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN  
ACCESSION NUMBER: 1993:142540 CAPLUS Full-text  
DOCUMENT NUMBER: 118:142540  
TITLE: Stabilization of enzymes by selective replacement of amino acids with proline



INVENTOR(S): Svendsen, Allan; Von der Osten, Claus; Clausen, Ib  
 Groth; Patkar, Shamkant Anant; Borch, Kim  
 PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.  
 SOURCE: PCT Int. Appl., 45 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9219726	A1	19921112	WO 1992-DK142	19920501
W: FI, JP, KR, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE				
EP 585285	A1	19940309	EP 1992-910123	19920501
EP 585285	B1	19980812		
R: AT, BE, DE, DK, ES, FR, GB, IT, NL, SE				
JP 06507071	T	19940811	JP 1992-509154	19920501
AT 169678	T	19980815	AT 1992-910123	19920501
ES 2121854	T3	19981216	ES 1992-910123	19920501
US 5914306	A	19990622	US 1993-140008	19931022
PRIORITY APPLN. INFO.:			EP 1991-610035	A 19910501
			WO 1992-DK142	W 19920501

AB Enzymes are made more stable by methods that minimize the structural flexibility of the peptide backbone. This is achieved by substituting selected amino acids with proline in regions of the protein outside stable higher order structures such as  $\alpha$ -helices and  $\beta$ -sheets. The criteria for selecting amino acids to be substituted also include dihedral angles  $\psi$  and  $\phi$  in the ranges -  $90^\circ < \phi < -40^\circ$  and  $-180^\circ < \psi < -150^\circ$ ,  $-80^\circ < \psi < 10^\circ$ ,  $100^\circ < \psi < 180^\circ$ . The invention is intended for use in the preparation of stable enzymes for use in detergents. Genes for a set of derivs. of the lipase of *Humicola lanuginosa* with individual amino acids substituted by proline were prepared by PCR mutagenesis of the gene and the expression cassette used to manufacture the protein in *Aspergillus*. An analog of the lipase with Gly225 replaced by Pro lost 50% of its activity after .apprx.30 min at  $70^\circ$  whereas the wild type lost 50% of its activity after 15 min. at the same temperature

=> log y